

Precision viscometry of extremely dilute solutions of high polymers

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With 17 figures in the text

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Preface

The present investigation was carried out at the Institute of Physical Chemistry of the University of Uppsala during the years 1954-1957.

I wish to express my deep gratitude to the Director of the Institute, Professor Stig Claesson, for suggesting this work to me and for his continuous interest, encouragement and helpful advice in frequent discussions and for his constant efforts to place the greatest possible facilities at my disposal.

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Introduction

In recent years many attempts have been made in several laboratories to establish the viscometric behaviour of solutions of high polymers of different types at very low concentrations. It is of special interest to investigate if there is an observable change in this behaviour in the interval of the concentration, where the molecules lose direct contact with each other. This case has been studied theoretically; in particular by Simha (1) and Riseman and Ullman (2). Experimental studies have also been reported by Rothman, Simha and Weissberg (3), Takeda and Turuta (4), Streeter and Boyer (5), Umstätter (6), Kooy and Hermans (7), Tuijnman and Hermans (8) and others.

During 1950-1952 an apparatus for the accurate measurement of viscosity was designed and built at this Institute by Claesson and the present author (9). Here the experimental technique and some results of viscosity measurements of solutions of high polymers at extremely low concentrations are reported. During the course of this work it was sometimes desirable to modify the original design of the apparatus. Previously short reports on this research program have been published (10-12).

The work is presented in four parts. The first part briefly presents the theoretical and experimental background of the work. In the second part the viscometers used are described and the common errors of the viscometric measurements are discussed. The third part includes mainly the preparative work and the actual measurements of the viscosity. Finally in the fourth part the results of the measurements are presented and discussed.

Uppsala, March 1958

PART I

THEORETICAL AND EXPERIMENTAL BACKGROUND FOR THE PRESENT WORK

Chapter 1. Some theoretical aspects concerning viscosity at very low concentrations and the conception of "critical concentration"

Simha (1) and Riseman and Ullman (2) have discussed theoretically the dependence on concentration of viscosity in dilute solutions. The result obtained indicates, that in dilute solutions of chain molecules the numerical value of k' in Huggins' equation

$$\eta_{sp}/c = [\eta] + k'[\eta]^2c \quad (1)$$

ought to be of magnitude 0.6–0.8 instead of 0.3–0.4 as is usually found experimentally. It is possible that these lower values are due to the fact that in general the measurements can only be made at such high concentrations that the assumptions given at the theoretical discussion are not valid.

Various attempts have been made to calculate the "critical concentrations", at which the polymer molecules lose direct contact with each other. Boyer and Spencer (13) have proposed the following definition of the "critical concentration". "The critical concentration" is the concentration corresponding to the hexagonal close-packing of spheres with diameters two times those obtained for the random coils of the molecules in question in the actual solvent. The factor *two* is introduced arbitrarily. This definition means that for polystyrene with $M = 10^6$ the "critical concentration" in benzene would be 0.0003 g/ml. But the hydrodynamically effective sphere is greater than the geometric volume the molecule occupies. Thus it may be supposed that the effects of the "critical concentrations" will take place at lower concentrations if hydrodynamic methods of measurements are used. Doty and Steiner (14) and others also found that for measurements with static methods the "critical concentrations" ought to be surprisingly high. (Many of these questions are discussed by Frisch and Simha in the monograph on Rheology, edited by Eirich (15).)

As to which solution of polymer is most suitable for an examination of these facts, the following may be said. The "critical concentration" increases with decreasing molecular weight, but as the specific viscosity probably decreases at the same rate it appears as though the specific viscosity, at which the "critical concentration" effects may be observed, is rather independent of molecular weight. In the same manner it may be independent of the type of solvent, good or poor. But there are yet some factors to be considered. Measurements with solutes of relatively low molecular weight are often easier to perform. On the other hand it is possible that in the change from a purely hydrodynamic interaction between the molecules to an interaction due

to direct contact when the "critical concentration" is passed, the change of energy dissipation is greater for larger molecules than for smaller ones. Thus the larger molecules will give a greater change in the viscosity curve at this concentration. It is also possible that this effect will be even greater for solutions in poor solvents since, in this case, the molecules are more compact and stiff.

Chapter 2. Earlier results of measurements of viscosities at very low concentrations

Some time ago it was reported (16, 17), that sometimes in a diagram of η_{sp}/c vs. c a linear relationship was obtained down to a rather low concentration, but at still lower concentrations there was a "bending down" of the curve. During recent years however the results obtained with apparatus especially designed for the investigations of this phenomenon have been particularly divergent. Rothman, Simha and Weissberg (3) found for polystyrene in toluene a linear relationship down to the lowest concentrations at which measurements could be made. Takeda and Turuta (4) found that the curve in question "bent upwards". They worked with polyvinyl chloride in cyclohexanone. Approximately the same result was obtained by Streeter and Boyer (5) with polystyrene in toluene and methyl ethyl ketone. This effect was assumed by Streeter and Boyer to be due to the entangling of the polymer molecules at these low concentrations. They also concluded that Rothman, Simha and Weissberg, using a viscometer of the dilution type, did not find this "bending up effect" because there was not time enough after the dilution in the viscometer for the molecules to disentangle and expand to a new position of equilibrium. But Streeter and Boyer also found that the curve after "bending up" reached a maximum and then tended to go down very abruptly at still lower concentrations. This was explained as an adsorption effect similar to that described by Cutler and Kimball (18); the concentration was diminished in the viscometer due to the adsorption of polymer molecules on the walls of the viscometer. Results similar to those of Takeda and Turuta and Streeter and Boyer were also obtained by Umstätter (6) with measurements on polyisobutylene (Oppanol) in decalin. Batzer (19) found that branched polyesters in different solvents gave a minimum-maximum curve in contrast to linear polyesters with which he obtained a linear relationship down to a relatively low concentration, and then there was a "bending down". He also found the same type of curves for polyvinyl chloride, branched and linear, in tetrahydrofuran. Menčík and Láníková (20), on the other hand, did not succeed in proving such a difference in the viscometric behaviour of solutions of branched and linear molecules, respectively. They also used polyvinyl chloride in cyclohexanone and tetrahydrofuran. In two of the preliminary reports by the present author (11, 12) these anomalous viscosity effects were discussed in terms of the thickness of the adsorbed macromolecular layer and reduction of capillary diameter. Kooy and Hermans (7), with an apparatus with the highest precision previously used, got a linear relationship for polystyrene in toluene down to the lowest concentrations. Tuijnman and Hermans (8), with the same apparatus as Kooy and Hermans but with some improvements, worked with polyvinyl acetate in toluene. They concluded that "in the system PVA-toluene the anomalous viscosity-concentration curve is due primarily to non-Newtonian effects".

Some of the works here mentioned will be commented on later in connection with the results now obtained (pp. 424, 432).

PART II

VISCOMETERS AND ERRORS OF VISCOMETRIC MEASUREMENTS

Chapter 3. Construction of the precision viscometers used

A. General considerations

From the previous measurements of the viscosity of high polymer solutions there is no doubt that, if there shall be any chance at all to solve the actual problem of viscosity, one must be able to measure a specific viscosity as low as 0.01 with an accuracy of at least one per cent. This also means that the viscometer totally must have an accuracy of at least 1 part in 10,000. But as the final error in the measurement is composed of errors from different sources, it is probable, that one ought to demand that the accuracy at each individual source of error be at least 1 part in 100,000.

B. A general survey of the viscometers used

During the construction of the viscometer it was also obvious, that it was only by using a capillary viscometer that the desired accuracy of 1 part in 100,000 could be obtained in each individual source of error. First of all an Ostwald viscometer was constructed. This construction was essentially based on the results published by Jones *et al.* (21-23) from some precision viscometers constructed in the 1930's. The real viscometer had the following dimensions and constants:

Efflux volume, 20 ml	Capillary diameter, 0.44 mm
Mean hydrostatic head, ~ 20 cm	Efflux time for water (25°C), ~ 1100 s
Capillary length, 24 cm	

Later an Ubbelohde viscometer was also constructed. It had about the same dimensions as the Ostwald viscometer.

In the recent years the apparatus has been rebuilt thoroughly, and two new viscometers have been constructed. The principal difference between these viscometers and the older ones is, that now the liquid in the viscometer is not pressed into the upper viscometer bulb as earlier, but comes to that bulb, when we tilt the whole viscometer. In that way we can have the viscometer totally closed when working with it, which can be of great importance: no evaporation and avoidance of dust.

Fig. 1 shows the apparatus in the present state.

A total view of the apparatus before the last great rebuilding may be seen in a paper by Claesson (9).

C. The construction in detail

In the description of the apparatus we have discussed, in part, some of the errors which may occur in the viscosity measurements and in this way some of the principles used in the construction of the viscometers become evident. It is also necessary to discuss some of the details of the apparatus in the next chapter, which deals with experimental errors.

1. The Ostwald viscometer

The modified Ostwald viscometer now used is shown in Fig. 2. The liquid is brought to the upper bulb A by tilting the whole viscometer (in the direction of the arrow).

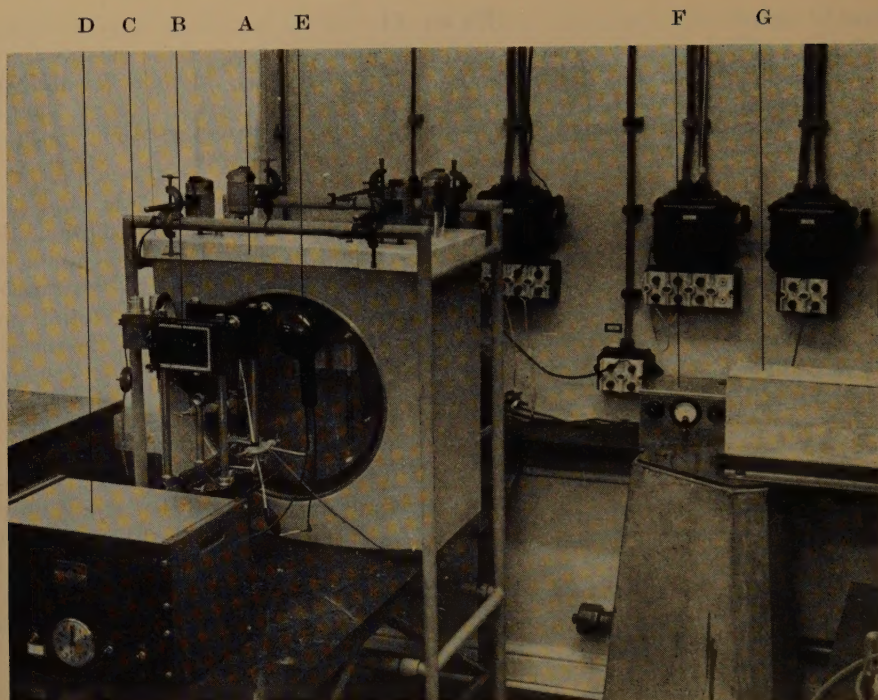


Fig. 1. High precision viscometer. *A*, thermostat; *B*, camera; *C*, clock; *D*, vacuum precision fork; *E*, electronic flash lightening tube; *F*, electronic control unit; *G*, casing for galvanometer and photo tube arrangement.

In doing so the liquid will first be emptied from the lower bulb *B* to a reservoir *C*, and then with further tilting it runs into *A*. The liquid left in the capillary from the preceding run will now be forced up into *B*. The viscometer is held in this inverted position for some time after *A* and the whole capillary are filled, so that as much as possible of the liquid remaining on the walls of *B* will run out of *B*. It is not tilted back until it has been in that inverted position long enough so that the variations of the mass of the liquid in the remaining liquid film do not influence the volume in *B* too much in the following measurement. If this is not the case the height of the liquid in *B* is changed so that the maximum error allowed in the hydrostatic head will be exceeded. The "last" drop of the liquid will be hanging on the tongue *D* and will fall into *C*, when the viscometer is tilted back again. Here it is essential that the connection between the capillary and *B* is made in such a manner, that the part of the liquid film that tends to flow towards the capillary will not be so large, that one runs the risk of getting air bubbles enclosed in that position. When tilting the viscometer back it is first placed in a horizontal position (Fig. 2*b*). Then some of the liquid will be kept separate and stays between an arbitrary point on the lower part of the capillary *E* and the tip of the little bulb *F*, whereas the rest of the liquid runs into *C*. Afterwards the viscometer is turned carefully, so that a little of the liquid cut off flows away at *F*. This ensures, that the other meniscus comes to a fixed mark about

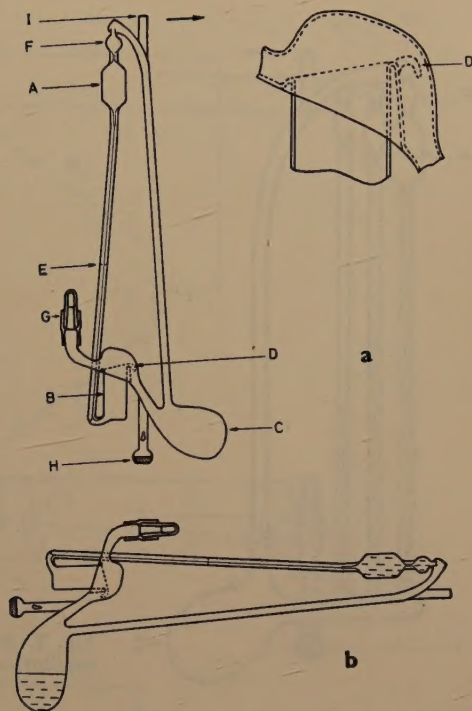


Fig. 2. Ostwald viscometer. (a) In position for measurement. (b) In position for adjusting of working volume.

the middle of the capillary *E*. This volume separated from the rest of the liquid will be used in the actual measurement of the viscosity. Then the connection between the capillary and the bulb *B* has such a shape that, when finally the viscometer is turned to the position for measurement, the irregular lowest part of *B* is completely filled with liquid when the bulb *F* is empty.

2. The suspended level viscometer

The suspended level viscometer is shown in Fig. 3. This viscometer has been constructed to permit simultaneous measurements to be made in three capillaries with different diameters. The reason for this arrangement will be given in the next chapter (see p. 413). There it is also pointed out that it is desirable to get about the same velocity gradient in all the capillaries. This has been obtained by holding the mean hydrostatic head about the same in all cases, whereas the wider capillaries have been made longer so as to give approximately the same maximum velocity gradient. To get similar efflux times for all the three capillaries, the upper bulbs have also been constructed with different sizes. The three different viscometers, which really constitute this suspended level instrument are in the following designated S1, S2 and S3, S1 having the widest capillary.

3. Some details common for both viscometers

The capillary of the Ostwald viscometer has the same diameter and length as that of S1 in order to get easily a comparison of the two types of viscometers. The corre-

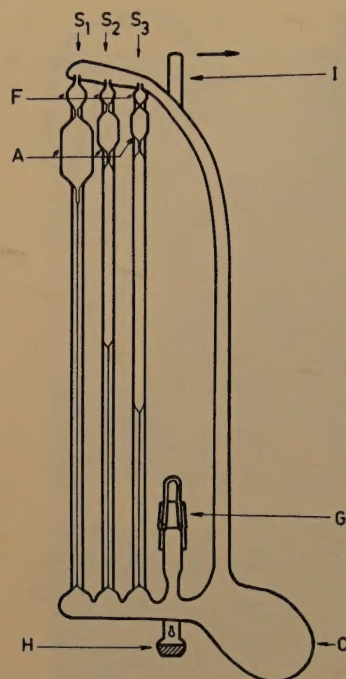


Fig. 3. Suspended level viscometer with three different capillaries.

sponding bulbs are also of about the same sizes. All capillaries are precision bored (Veridia). The ground glass joint *G* is made tight with a polytetrafluoroethylene sleeve (Longborough Glass Co.) and is shown in the figures with a rubber tube outside the cap (see also p. 419).

The viscometer is fixed in the holder in the thermostat by pressing the ball ground joint *H* by means of a spring (not shown in the figure) against a conical hole, while the tap *I* at the top of the viscometer is pressed against a V-shaped track in the holder by a piece of metal covered by a rubber sheet. Then to get the viscometer in its right position it is turned round its vertical axle so that the "backside" of the reservoir *C* is pressed against a metal head on the holder. In Table I some of the most important dimensions and constants of the viscometers are given.

4. The thermostat

When the temperature is changed, the viscosity of the liquids used as solvents for viscosity measurements of the type here described is changed about 1–2 per cent

Table I. Viscometer dimensions and constants.

Viscometer	Ostwald	S1	S2	S3
Efflux volume, ml	25	25	10	3
Capillary length, cm	40	40	24	17
Capillary diameter, mm	0.5	0.5	0.3	0.2
Flow time for water at 25°C, s	1460	1350	2340	1700

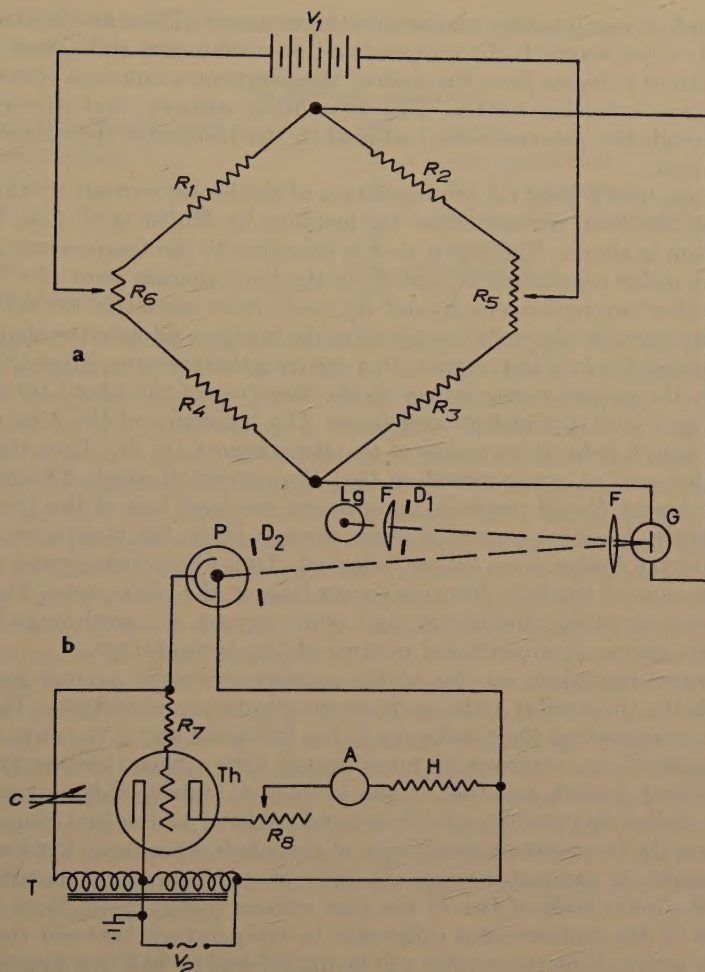


Fig. 4. Wiring diagram for temperature control unit. (a) Wheatstone bridge. (b) Electronic control unit.

per degree (C). (The change of the efflux time of a viscometer is about the same for a given liquid.) Thus to get a relative error of only $1/100,000$ a constancy of the temperature of better than $1/1000$ of a degree is required. Therefore we have done a great deal of work with the thermostat.

The thermostat vessel is made of aluminum, $70 \times 70 \times 30$ cm, and insulated with 5 cm of soft foam plastics. The cover is insulated with 5 cm of stiff foam polystyrene. In the front of the thermostat there is a round glass window (diameter 50 cm) insulated with two sheets of acrylate glass, placed so that two air layers (1 cm thick) are obtained, thus increasing the insulation effect.

For heating there are two heaters using constant current but also two more taking

current, which is regulated by a resistance thermometer. These last heaters normally generate only a few watts. If the temperature for some reason sinks more than about one hundredth of a degree from the desired temperature, a common contact thermometer activates a further heater. This precaution ensures, that abnormally high currents through the galvanometer included in the resistance thermometer wirings shall be avoided.

The principle that is used for the regulation of the heater current by the resistance thermometer, has been recommended for instance by Müller *et al.* (24). In Fig. 4 a wiring diagram is shown. The organ that is sensitive to the temperature in the thermostat is the nickel resistances R_1 and R_3 in the two opposite arms of a Wheatstone bridge; the other two resistances R_2 and R_4 , made from manganin are rather insensitive to temperature. At the right temperature the bridge is adjusted so that no current will flow through the null instrument, G , a mirror galvanometer. Then the light that is thrown by the galvanometer mirror in the direction of the photo tube P will fall only partly on P and diminish its resistance. The boundary of the area of the light falling on P is well defined by means of two diaphragms D_1 , D_2 . Thus the thyatron Th is fired for part of every period so that the current through Th and thus also through the heater H will precisely compensate the heat loss of the thermostat. If the temperature, for some reason or other, sinks a little, the resistances R_1 and R_3 diminish, and the bridge is no longer balanced. Thus the galvanometer deviates so that a little more of the light from its mirror falls on the photo tube. Then the thyatron fires earlier during the period, and more current will go through the heaters. This gives of course a proportional control of the temperature.

The electrical insulation on the nickel resistances should permit good thermal contact with the thermostat water and yet be completely watertight. This has been provided by constructing the resistances in the following way. On a thin-walled tube of brass (length 45 cm, diameter 18 mm) treated with a silicon lacquer the resistance wire was bifilarly wound, and then it was lacquered. Outside this unit another tube of brass, as narrow as possible, was placed and soldered to the first one at the lower edge. To raise the temperature sensitivity of the whole equipment further, the water of the thermostat is pumped through the inner tubes by means of centrifugal pumps applied at the lower ends of two of the four stirrers totally used. With the present construction of the resistances a difference in temperature between the resistance wire and the water of the thermostat will be diminished by half in a time shorter than a second. By having the area of the resistances and the length of the wires relatively great it is also sure that the temperature of the resistances will be only very little higher than that of the water, in spite of the heat which must be produced in the bridge.

Perhaps it should be pointed out that not only the nickel resistances but also the rest of the Wheatstone bridge are placed in the thermostat, as this is of course the simplest way to keep it as electrically constant as possible.

The coupling elements and other details included in the Wheatstone bridge and in the electronic control unit are listed below.

The Wheatstone bridge

R_1, R_3 1000 Ω nickel	R_6 5 Ω Helipot potentiometer
R_2, R_4 1000 Ω manganin	G galvanometer
R_5 100 Ω Helipot potentiometer	V_1 10 V lead storage battery

The wire of R_1 and R_3 is made from a very pure nickel quality (British Driver-Harris Co. Ltd), diameter 0.06 mm.

The electronic control unit

Th	Raytheon 2050. Two units in a parallel coupling	H	heater
P	RCA 1 P 37	T	auto transformer
C	0-50 pF	V_a	127 V ~ stabilized
R_7	1 M Ω	Lg	galvanometer lamp (white light)
R_8	0-1000 Ω	F	lenses
A	0-1 A amperemeter	D_1, D_2	diaphragms

As heaters two rod-shaped incandescent lamps (Skandia 4 B, 40 W, 220 V) are used, each connected in series with one of the thyratrons. As their heat capacity is very low, they react rapidly, if the current through them varies.

For control of the temperature a Beckmann thermometer is used. But it is also possible to control this by observation of the position of the light spot on the photo tube. Fluctuations of the temperature of very short duration are easy to follow, due to the fact that the nickel resistances change their values very quickly.

With such a proportional control of the temperature it is impossible, as was pointed out by Claesson (26), to be quite independent of the temperature of the room. A fall in the temperature of the room will immediately result in a drop of the equilibrium temperature of the thermostat. The sensitivity of the equilibrium temperature to the variations in the room temperature is of course for a given thermostat wholly dependent on the capacity of the thyatron to compensate a sinking of the temperature and thus on the maximum power obtainable from the same. In this actual case it is enough to hold the variations of the temperature of the room within six tenths of a centigrade degree by means of a simple arrangement for thermostating. The constancy of the position of the equilibrium temperature is of course in the same way dependent on the variations of the energy that is brought to the thermostat in other ways than by the control unit. Most important of these effects is the heat added by the constant heaters and the stirrers, and for this reason only stabilized current is used in the whole equipment.

5. *The measurement of the efflux time*

A photographic method is used for the measurement of the efflux time. A photograph is taken of the timing marks, above and below the upper viscometer bulb, and also on the same plate a running clock (via a mirror), just as the meniscus passes these marks. By means of a comparator the distance between the meniscus and the actual mark is measured. Then it is easy to correct the time given by the clock on the photographic plate to the time when the meniscus really passed the mark, if only the velocity of the meniscus is known. This velocity is obtained from a photograph with two exposures of the meniscus on the same plate taken in the course of a few tenth's of a second. In that way the efflux time will be determined in an objective manner with an accuracy that practically is limited only by the accuracy of the clock used.

In these measurements we use a Jaquet clock with synchronous motor works. It receives its alternating current from an oscillator, a Type 816 vacuum tube precision fork, General Radio Co., in which the thermostated fork presses its own frequency on the output voltage. The output power is two watts maximum and this is quite enough for the clock without any amplification.

The camera, designed with magazine for plates, 9×12 cm, is mounted on a heavy optical bench. A Zeiss Tessar Objective, $1:6.3$, $f = 10.5$ cm, is used. At a distance of 25 cm an image of the viscometer and the clock, with a reduction of 0.7, will be obtained. The photographic plate is Gevaert Gevachrome, 32° . In the actual photographing some different procedures are applied. Recently the light source has been a synchronized electronic flash lightening tube, Braun Hobby Standard with a power of 60 watt-seconds. The two exposures for the determination of the velocity of the meniscus are obtained by means of a special shutter in which a plate of metal with two slits slides past the objective lens with a suitable velocity at the same time as the viscometer and the clock are illuminated, each with a photographic lamp of 150 watts.

Chapter 4. Sources of errors. Accuracy and corrections of the viscometers used

A. General considerations and a list of the errors discussed

In the discussion of the errors of the viscosity measurements some of the most well-known errors in such measurements will be treated, but in addition some other errors will be discussed. These later are of two different kinds: errors of general importance for viscosity measurements and errors dependent on the special type of viscometers used here.

The viscosity measurements of the kind reported here are of course based on the modified Poiseuille's law

$$\eta = \frac{\pi r^4 h \rho g t}{8 V l} - \frac{m \rho V}{8 \pi t l}, \quad (1)$$

where η is viscosity, r radius, h hydrostatic head, ρ liquid density, g acceleration due to gravity, t efflux time, V efflux volume, l capillary length, and m a correction constant. When the errors are described here, it has been considered more suitable not to discuss the individual factors in this equation separately. Sometimes a special source of error has been observed and then its influence on the different factors in Poiseuille's law is discussed. It is perhaps suitable to point out here again that we have endeavoured to insure that all individual, relative errors do not amount to more than 1/100,000.

The following errors will be discussed:

- | | |
|----------------------------------|--|
| 1. Temperature | 6. Working volume (Ostwald viscometer) |
| 2. Time measurements | 7. "Kinetic energy" |
| 3. Drainage | 8. Barometric pressure |
| 4. Inclination of the viscometer | 9. Adsorption |
| 5. Surface tension | |

This classification of errors is not rigorous. Small errors in connection with the errors in one of the above groups are sometimes treated here, even though they do not belong to the group in question. Some accidental errors are very difficult to estimate theoretically. They will only be discussed in part 3 in connection with cleaning and preparations.

From the detailed discussion below it is obvious that it has been considered suitable to design the apparatus so as to reduce the errors Nos. 1-6 to within the permitted limits, whereas for the errors Nos. 7-9 corrections are usually applied.

B. Discussion in detail

1. Temperature

As already stated an accuracy in the temperature of better than $1/1000^{\circ}\text{C}$ is sometimes needed (the viscosity is changed by 1 to 2 per cent per degree and the density by 0.1 per cent). For this reason the resistance thermometer of the thermostat (p. 406) has been constructed so that a change in the temperature of $1/1000^{\circ}\text{C}$ will give a deviation of the galvanometer great enough to cause a change in the position of the light spot on the photo tube by about 3 mm (at a distance of 0.6 m). Then as the drifting of the zero position of the galvanometer usually does not amount to more than 1 mm during relatively long periods, there is no difficulty in maintaining the temperature with the required accuracy. In general the error seems to be not greater than $1/2000^{\circ}\text{C}$.

Besides the error in the temperature which depends on the thermostat there is another caused by the heat of friction in the viscometer. This occurs partly when the upper bulb is filled and partly in the capillary during the measurement. But after the filling of the upper bulb the viscometer is held in the inverted position for so long time, that the liquid surely adopts anew the temperature of the thermostat. Then during the measurement there is, with water as solvent, a difference in temperature of $1/1000^{\circ}$ between the two ends of the capillary, if we assume the process to be adiabatic (the corresponding difference for organic solvents is twice this value). But since we calculate that a certain amount of heat is lost through the capillary and since the variations in this temperature difference are small compared to the difference itself, this effect is negligible. It also seems possible to compare directly the results from the capillaries with different diameters in spite of different temperature increments.

Finally it may perhaps be said that the influence of the temperature on the dimensions of the viscometer is of little importance in this connection.

2. Time measurements

The vacuum tube precision fork used in the equipment for the measurements of the efflux time has a stability of the frequency guaranteed to be at least 1 part in 100,000. This has been checked over long intervals against the standard time signal. The fastest hand of the synchronous clock, which makes one turn per second, has also been checked to see if its speed is the same during the whole turn. This has been done by taking photographs of the running clock with the fastest hand in ten different positions and on the same plates a scaler. These checks demonstrated that the clock had the accuracy required.

The efflux times for the solvents used have not usually been much shorter than 1000 seconds. In this case the accuracy required is $1/100$ of a second. On the photographic plates (p. 407) it is easy to decide the position of the fastest hand with better accuracy than $1/100$ of a second. When the Ostwald viscometer and the S1 viscometer are used, the velocity of the meniscus, when passing the timing marks, is such that 1 mm on the plate corresponds to a time of 3–6 hundredths of a second. For the S3 viscometer however the velocity of the meniscus is only about a tenth of that for S1, and to get the same accuracy again in the correction it is necessary to measure the actual distance within some hundredths of a mm; this is usually quite easily done with the comparator. (A reduction of the diameter of the capillaries above and below

the upper viscometer bulb to get higher velocities has not been considered as advantageous. A production of small drops would easily take place in the upper capillary.)

3. *Drainage*

According to Jones and Stauffer (22) the drainage in a capillary viscometer is approximately independent of the kinematic viscosity if the liquid flows only under the influence of its own weight. Of course it is claimed that the measurements are always made in the same manner. Thus it is necessary, for instance, that the liquid is raised to the same height above the upper viscometer bulb, so that the drainage always will come from the same surface. This will be done automatically in the viscometers used here, as the additional little bulb *F* (Figs. 2 and 3, pp. 403 and 404) is always filled before the measurement and it is impossible for the drainage from outside to enter it.

The drainage in the lower bulb of the Ostwald viscometer must also be observed. This will of course not change the efflux volume but only the hydrostatic head. In this case it is made as small and as reproducible as possible by holding the viscometer in the inverted position a relatively long and definite time (15 min). From tests it seems as if the total drainage for the lower bulb does not reach the maximum error of the volume that is allowed.

4. *Inclination of the viscometer*

As the Ostwald viscometer is of the oblique type with the two viscometer bulbs directly above each other, and as the mean hydrostatic head is approximately the same in all cases for both viscometers, we almost always must have the same error due to the inclination of the viscometer. Thus with a hydrostatic head of 40 cm and with, for instance, the lower meniscus fixed, the maximum variation in the upper bulb should not be greater than 3 mm in any direction. But actually the maximum displacement allowed is surely determined by the error in aligning the upper bulb above the lower during the construction of the viscometer. An error of about 5 mm in this alignment would diminish the maximum displacement described above to only $\frac{1}{3}$ mm. Such errors have not been exceeded in this work.

5. *Surface tension*

The errors dependent on surface tension are among the most difficult to overcome. To eliminate the effect completely for troublesome systems (water solutions of surface active substances for instance) by constructing the apparatus in a suitable manner seems to be practically impossible. Different methods to correct for the effect have been worked out both on theoretical and experimental bases, but usually they are most difficult to use (23, 25). In this work however the Ostwald and the suspended level (S1) viscometers must have different signs for the error obtained due to the dimensions of the different menisci, and then it is of course relatively easy to decide if corrections are necessary. For the organic solvents used they seem to be negligible.

6. *Working volume (Ostwald viscometer)*

With the construction used here where the working volume intended for a measurement is determined anew for every individual flow and is only a part of the liquid existing in the viscometer, the volume of this liquid may of course be varied within very wide limits. The maximum error allowed in the actual working volume may be

2 mm³. This error is determined by the position of the meniscus at the mark on the capillary *E* (see Fig. 2*a*, p. 403) and at the tip of *F*. An error as great as 0.5 mm at *E*, and which is surely never exceeded, gives an error in the volume of only 0.1 mm³. The error at *F* could easily be greater but it never seems to amount to as much as 1 mm³. During tests it has been possible to make the total error smaller than 0.2 mm³.

7. "Kinetic energy"

The "kinetic energy" correction of the specific viscosity η_{sp} is determined approximately for diluted solutions by the following formula derived by Schulz (27):

$$\eta_{sp} = \{\eta_{sp}\} (1 + 2 B/t_0^2). \quad (2)$$

Here $\{\eta_{sp}\}$ is the uncorrected value of the specific viscosity and *B* is a constant characteristic of the apparatus (see below). Thus when the kinetic energy correction is applied to the primary values of the specific viscosity, a plot of η_{sp}/c versus *c* will be a curve parallel to but displaced upwards from the curve obtained when the uncorrected values were used. In an investigation such as this, which primarily deals with the shape of the curve and not the value of $[\eta]$, the error due to the "kinetic energy" has, contrary to what has been occasionally asserted, a diminutive importance by itself. On the other hand, due to the importance of the position of the experimental curve when we want to discuss the adsorption of polymer on the walls of the capillary, it is very important to be able to determine the "kinetic energy" correction with great accuracy. This adsorption will, in certain cases, have a great influence on the shape of the curve.

None of the methods for the determination of the "kinetic energy" correction usually recommended in the hand books of viscosity has been considered as adequate in its original form. But by modifying one of these methods in a suitable manner a satisfactory result has been obtained.

Poiseuille's law corrected for the "kinetic energy" error (Eq. (1)) may be written as

$$\eta = \varrho A (t - B/t) \quad (3)$$

where *A* and *B* are constants of the apparatus, and the term $\varrho A (B/t)$ is the "kinetic energy" correction. If the values of *A* and *B* are known, it is possible to calculate the viscosity of a liquid from its flow time. To measure *A* and *B* it is usual to determine the efflux times for two liquids with known viscosity and density, and then to calculate *A* and *B* from Eq. (3). When evaluating the constants we get (indices 1 and 2 refer to liquid 1 and 2 respectively, $\nu = \eta/\varrho$ is the kinematic viscosity):

$$A = \frac{\nu_2 t_2 - \nu_1 t_1}{t_2^2 - t_1^2}, \quad (4)$$

$$B = t_1 t_2 \frac{\nu_2 t_1 - \nu_1 t_2}{\nu_2 t_2 - \nu_1 t_1}. \quad (5)$$

But the primary aim of this work is to determine relative viscosities and not absolute ones and hence it is not necessary to know the value of *A* but only that of *B*. From Eq. (5), which may be written as

$$B = t_1 t_2 \frac{t_1 - t_2 (\nu_1/\nu_2)}{t_2 - t_1 (\nu_1/\nu_2)}, \quad (6)$$

it is possible to calculate B from the flow times of two liquids if we only know the quotient between their kinematic viscosities. But for a capillary viscometer, where the "kinetic energy" correction may be neglected:

$$\nu = \text{constant} \times t, \quad (7)$$

and thus it is possible to determine the quotient between the kinematic viscosities of two liquids by measuring the flow times in such a "correction free" viscometer. There is no great difficulty in constructing capillary viscometers with arbitrarily small "kinetic energy" corrections, *if they are only designed to be used for occasional measurements with low molecular liquids*. Such viscometers will necessarily have very long efflux times. This fact among others causes that they are not convenient for common use.

In connection with some measurements to estimate the influence of shear rate on the viscosity results obtained, a new viscometer was recently constructed (see p. 426). This is of the Ubbelohde type, and as it gives very low "kinetic energy" errors and is rather easy to work with, the last determinations of B have been made by means of this. Its dimensions and constants are: Capillary lengths 150 cm, radius 0.15 mm, driving head ~ 30 cm, relative error due to "kinetic energy" $\sim 1/100,000$ for water.

Among the advantages of this modified method the following will be mentioned. If the values of the viscosity and density of a liquid are taken from works of reference, it is necessary to be certain that the preparation of the liquid used for the calibration is exactly the same as that used by the observer in the reference. With this new method on the other hand, the calibration liquids need only be free of dust. Thus we are free to choose reference liquids as we please, and it is possible for instance to choose a mixture of two liquids or more. In practice the dimensions and properties of a viscometer will vary a little with for instance different surface tensions (and thus A and B are not real apparatus constants, if we do not take the surface tensions into consideration). It may then be convenient in the determination of B to use partly the solvent to be used later and partly one or still better some liquids with other viscosity values. But it is very convenient if not only the surface tension of these liquids but also other physical properties, which may influence the efflux time, are as similar to those of the solvent as possible. Then for measurements on macromolecular solutions it may be of importance that the quotient of the efflux times, from which the constant B is calculated, only depends on the viscometer and the viscosity of the liquids.

8. Barometric pressure

As has been pointed out by Kooy and Hermans (7) the efflux time of the viscometer depends to some degree on the atmospheric pressure. The main error is caused by changes in the density of the air. It is relatively unimportant; for toluene a change of 5 mm Hg in the air pressure gives a change of the efflux time of about 1/100 second if the total efflux time is 1000 seconds.

It may be appropriate to point out that, for the viscometers which are totally enclosed in these experiments, a pressure error also may arise if there are temperature gradients when the viscometer is closed. This error is caused by two factors. Firstly due to the direct pressure increase with temperature (a difference of the temperature of 2° will give a pressure error of just about 5 mm Hg) and secondly to the change of the vapor pressure of the liquid in the viscometer when the temperature is changed. Both errors are of about the same magnitude when the "worst" liquids in this work

are used. These errors work in the same direction but, as the filling and the closing of the viscometer are always made in the room where the thermostat is placed and this room is thermostated to better than one degree (see p. 407), there is no risk that this temperature-pressure error will interfere with the measurements reported here. (When the viscometer is closed the air in the viscometer is always saturated with the vapor of the liquid used, see p. 419; if not, another pressure error would arise.)

9. Adsorption

The adsorption of polymer from the solutions used in the measurements causes at least three different errors.

Usually the concentration of the solution is determined by weighing, but then when polymer is adsorbed on the walls of both the bottles used and the viscometer, the concentration is decreased and the efflux times determined are too short when compared with the original concentrations (18). Thus the values of η_{sp}/c will be somewhat low. This error may be rather marked at very high dilution. To a certain degree it may be avoided if the viscometer is shaken with a part of the solution before the measurement. In a similar way it may also be reduced during the preparation of the solutions (see p. 418).

Another error due to adsorption is caused by the diminishing of the diameter of the capillary when the polymer is adsorbed. This error may greatly change the shape of the curve η_{sp}/c vs. c . The importance of this error has arisen in that work, but as two preliminary reports on the subject have already been published (11, 12), it will be discussed but briefly here.

It must be assumed that the molecules adsorbed to some degree at least retain the volumes and the shapes they have in the solution, if the reduction of the diameter of the capillary shall be great enough to change the efflux time to measurable extent. To calculate the influence of the high polymer layer adsorbed on η_{sp}/c we denote the apparent relative viscosity observed by η_r^* , the true relative viscosity by η_r , the capillary radius r and the thickness of the layer by a . Then we have

$$\eta_r = \eta_r^* \left(\frac{r-a}{r} \right)^4 = \eta_r^* (1 - 4a/r) + \dots, \quad (8)$$

which gives

$$\eta_{sp}^*/c = \eta_{sp}/c + 4a (\eta_{sp}/c + 1/c) \cdot 1/r, \quad (9)$$

where η_{sp}^*/c is the apparent reduced viscosity and η_{sp}/c the true one.

For a given concentration a may be assumed constant and then, due to Eq. (9), we have a linear relationship between η_{sp}^*/c and $1/r$. Thus if we measure the viscosity at this concentration with capillaries with different diameters and plot η_{sp}/c against $1/r$, the intercept will give the true η_{sp}/c since the adsorbed layer at $r = \infty$ does not influence the efflux time. The suspended level viscometer has been constructed with three capillaries with different diameters (see p. 403) in order to extrapolate to $r = \infty$. (A similar Ostwald viscometer, fairly convenient when used, seems to be very difficult to construct.) The extrapolation is only permitted when the liquids are Newtonian, or if we work at about the same maximum gradient of velocity in all the capillaries. As mentioned, this latter condition has been arranged during the construction of the suspended level viscometer (p. 403). Thanks to this same maximum

gradient in all capillaries the mechanical deformation of the adsorbed layer due to the flowing liquid is very likely the same.

Since the publication of the preliminary reports on this adsorption error, further publications on the same subject have become available. They will not be discussed here, but in the last part of this work many of them will be discussed in connection with the presentation of some new results concerning this problem (p. 432).

The last error due to adsorption refers to the volume. The adsorbed layer will diminish the volume of the upper bulb. This error is not great but will perhaps now and then influence the results. Relatively it may be of the magnitude $1 - 2 \times 10^{-5}$ for S1 and about the double for S3. See also p. 426.

PART III

PREPARATIVE WORK AND THE ACTUAL MEASUREMENTS OF VISCOSITY

Chapter 5. General considerations

For our measurements we have used mainly two different types of molecules. (1) Statistically jointed chain molecules of the same kind but with different molecular weights. (2) Rigid linear macromolecules of different molecular weights in a good solvent to ensure that they are as elongated as possible.

As a suitable representative for the first polymer type polystyrene was chosen for several reasons. It is relatively easy to polymerize styrene and to fractionate the polymer obtained. Furthermore polystyrene is quite easy to work with, a fact which is very important in this case since the measurements in themselves are intricate. It also seems to be one of the most well-characterized synthetic high polymers and hence it is easy to make comparisons of different kinds; several of the works already published dealing with the problems in view are all made with polystyrene (3, 5, 7). As solvent toluene was chosen; this was also used in the references given above.

As a suitable polymer with rigid linear molecules nitrocellulose was chosen with ethyl acetate as the solvent.

Besides the measurements with the systems mentioned some other measurements have also been made. Linear polymethyl methacrylate was compared with a branched copolymer of methyl methacrylate and glycol dimethacrylate with ethyl acetate as solvent. Recently two different types of polyvinyl acetate, one linear and the other branched, were also investigated.

Chapter 6. Preparative work

The preparative work mainly consisted of making polymerizations and fractionations. For the characterization both of the crude polymers and the fractions, determinations of the intrinsic viscosities were made. Sometimes the sedimentation constants were also determined.

A. Polystyrene

1. Polymerization

Only some of the measurements with polystyrene will be discussed in detail in this paper and, for this reason, only the polymerization of styrene will be described exhaustively, whilst the work with the other synthetic polymers will be but briefly mentioned.

The styrene polymerization was made in bulk with benzoyl peroxide as initiator. The main objective in the preparation was to get fractions with different molecular weights in a rather wide interval and with the structure of the molecule as homogeneous as possible throughout all fractions. To do this it was considered best firstly to polymerize with a low concentration of initiator and at a low temperature and secondly to stop the polymerization at a relatively low degree of conversion.

The styrene used (Kebo, purum, 99–100 %, stabilized with *p*-tert-butylcatechol) was first set free from the inhibitor by washing twice with 10 % sodium hydroxide. Then it was washed three times with distilled water and dried with Drierite (white). Finally it was distilled twice in a column in an atmosphere of nitrogen at low pressure (70 mm Hg). The receiving vessels were cooled with ice.

The benzoyl peroxide used ("Benzoylsuperoxid, reinst, f. wissenschaft. Zw. Dr. Theodor Schuchardt, München, Germany") was first recrystallized from chloroform (LKB, pro analysi). Then it was dissolved in acetone (Merck, pro analysi), and sufficient distilled water was slowly added to cause about half of the peroxide to reprecipitate. The precipitate was placed on a fritted glass filter and dried in vacuum at room temperature.

The middle fraction obtained in the second, relatively rapid distillation of the styrene, was distilled directly into a glass ampoule with 2.3 cm inside diameter and with a length of 42 cm. It was filled to about $\frac{9}{10}$ of its volume. The total amount of benzoyl peroxide was calculated (with respect to a suitable degree of conversion) to be only 4.71 mg. Since its weight ought not to differ too much from this value, it was suitable to measure this amount volumetrically. The correct portion of a dilute solution (in methyl ethyl ketone) with known concentration was introduced in the ampoule. The solvent was evaporated after which the styrene was distilled (the ampoule was then cooled with ice as already mentioned), and finally the ampoule, cooled with dry ice and evacuated (about 0.5 mm Hg), was closed. When the frozen styrene had melted again the ampoule was shaken a little until the peroxide was dissolved and put into a thermostat with a temperature of 58°C. With the thin ampoule the temperature of the polymerizing system ought not to have risen appreciably above the temperature of the thermostat. The polymerization time, 55 hours, had been chosen so that a degree of conversion of only about 10 % was to be obtained. To stop the polymerization the polymerizing system was poured into cooled methanol,

Table 2. Polymerization data for polystyrene.

Styrene, weight in g	155.6
Polystyrene, weight in g	13.4
Degree of conversion, %	8.6
Benzoyl peroxide, % by weight	0.003
Temperature of polymerization, °C	58
Time of polymerization, hours	55
Intrinsic viscosity, $[\eta]$, of the crude polymer, ml/g (in toluene)	280

so that the polymer was precipitated, and then almost immediately the precipitate was taken on a filter and carefully washed with methanol. The precipitate was dissolved in methyl ethyl ketone (5%) and the solution, while being stirred, was slowly poured into the same volume of methanol and after 2 hours the precipitate was taken on a filter, washed with methanol, and dried in vacuum at room temperature.

A comprehensive list of data for the polymerization is given in Table 2.

2. Fractionation

Here, for the same reason as in 1, only the fractionation of polystyrene will be described. The method of precipitation was used. It seemed best to get the polystyrene (~13 g) obtained in fractions, the weights of which should not be considerably lower than 0.5 g and not higher than 1.5 g. About twenty fractions were desired. Thus the weight should normally be lower than 1 g. To get a result as good as possible with a reasonably small amount of work it was decided to use successive precipitations according to the principle recommended for instance by Flory (28) and Boyer and Simha (29). In each precipitation from the main solution a somewhat larger fraction than desired is obtained and this fraction is then redissolved in a rather large amount of solvent and precipitated again to a suitable degree from a more dilute and thus also a more appropriate solution than the main one. When that second precipitate is filtered off the rest of the polymer in this dilute solution is also precipitated and returned to the main solution to which a little solvent is added so that all the polymer redissolves. This method permits one to work with small volumes but nevertheless the fractions are obtained from very dilute solutions.

Methyl ethyl ketone was used as solvent and methanol as the precipitating agent. An original concentration of the main solution of about 0.5% was considered as suitable; in all 2.7 l methyl ethyl ketone was used. When methanol (330 ml) had been added to the solution until a slight turbidity was obtained, the solution was heated so that the temperature rose from 25° to about 35°. An additional 12 ml of methanol (the amount determined from a previous experiment) was added and then the solution allowed to cool slowly (during ~10 hours) to 25°. The solution was stirred during this time. At 25° the stirring was stopped and the precipitate was allowed to settle.

In fractionation work we always worked with enclosed flasks in order to avoid vaporization. For the stirring in these flasks a special stirrer, described in another paper (30) was constructed. It functioned satisfactorily, and especially the simple shape of the part dipping into the liquid, in reality consisting of a straight glass rod, was important as almost no precipitate fastened to the same. Only when precipitating the last low molecular weight fractions was there any indication of polymer on the rod, but this was easy to remove.

Table 3. Fractions of polystyrene.

Fraction No.	Weight g	$[\eta]$ ml/g (toluene)	$\bar{M}_v$
4	1.1	470	2,500,000
12	0.5	214	800,000
18	0.4	118	300,000

When a few fractions had been obtained it was necessary to increase the amount of methanol added per fraction to get a suitable amount of the precipitate. In the same manner the temperature must be increased to 40° in order to prevent precipitating when the methanol was first added. In all, 20 fractions were obtained. For their characterization their intrinsic viscosities were determined. For this work an Ubbelohde viscometer of the dilution type was used. Weights and intrinsic viscosities of the three fractions used in the following measurements are given in Table 3.

B. Nitrocellulose

The nitrocellulose used was kindly placed at our disposal by Dr. A. G. Chitale. Besides the viscosity measurements made by the present author some other measurements both by Dr. Chitale and others have been made with these same samples at this Institute. It was considered best to make measurements partly with a rather highmolecular and partly with a more normal fraction.

The nitrocellulose was prepared by cotton nitration. A determination of nitrogen content gave the value 13.87 %. (The analyses were carried out at the Central Analytical Laboratory, Uppsala University.) For the fractionation the precipitation method had been used. Acetone was the solvent and water the precipitating agent. The fractions were dried in vacuum (nitrogen atmosphere) at ~60°C. Some data for the fractions used here are given in Table. 4.

Table 4. Fractions of nitrocellulose.

Fraction No.	$[\eta]$ ml/g (ethyl acetate)
2	880
6	405

Chapter 7. Measurements of the viscosity

A. Preparation of the solutions

1. Polystyrene

After the preparation, the polystyrene was stored for several months in a vacuum desiccator. The last 24 hours before the solutions were prepared the desiccator was evacuated with a forepump to a vacuum less than 1 mm Hg. A trap between the pump and the desiccator was cooled with a mixture of dry ice and chloroform.

The solvent was redistilled about a week before it was used. In that way variations of its properties with time surely should have passed the relatively rapid initial stage before the solutions were prepared. It was desirable to keep the moisture content rather constant and of a relatively low value. Before the distilling the solvent was first dried carefully (with Drierite) and afterwards stored in special flasks from which suitable portions could be pressed out with dry air. (This operation lasted for less than a minute and the additional pressure was slight so that the risk of dissolving much air was negligible.) Further intricate procedures to avoid absorption of moisture from the air during the preparation of the solutions were not made.

The concentrations of the solutions were usually determined by weighing. The

most diluted solutions were obtained by dilution from stock solutions in one or two steps. To reduce the concentration error, due to adsorption, as much as possible for the most diluted solutions where its effect may be rather great, the dilutions were made in the following way. Usually the last 5 to 6 solutions were obtained from the same stock solution. This solution was first divided into 5 to 6 equal parts and each poured into a weighed separate flask. The flasks were then shaken carefully from time to time with the solutions stored in them. After 3 to 4 hours all solutions were poured back into the stock solution flask and shaken together. Then adequate portions of the stock solution so treated were poured into the flasks already rinsed with the solution, weighed and diluted with pure solvent. This procedure changed the concentration of the stock solution a little (evaporation and adsorption working against each other), but at the same time the concentration was almost exactly the same for the stock solution portions in the different flasks, and there were no adverse adsorption effects when most dilute solutions were prepared.

Both theoretical determinations and experimental test showed that the errors in the concentration apart from those due to adsorption were barely higher than $\frac{1}{2}\%$ at any time, and usually they were much lower. By following a fixed procedure for the preparation of the solutions the errors were probably rather equal in value for the different solutions and of the same sign. Thus their importance for the measurements in question was largely eliminated; a pervading, smaller error in the concentration will not change the shape of the curve η_{sp}/c vs. c very much but only its position. Usually the volume of the solutions prepared was about 100 ml. The solvent was added the polystyrene and left for twenty-four hours, during which time it was shaken repeatedly. The final diluted solutions were also not used for twenty-four hours. After preparation, the solutions were stored in closed flasks in a box where the moisture was removed with calcium chloride. Usually some ten solutions were prepared for every measurement on a fraction. The concentrations were chosen so that the most diluted one gave an η_{sp} of ~ 0.001 , whereas the concentrations of the rest usually were distributed to give η_{sp} between this value and 0.1. The pure solvent used in the series of measurements to obtain t_0 (efflux time of solvent) was manipulated in a similar way and stored in closed flasks as were also the solutions.

2. Other polymers

Usually the solutions of the other polymers were prepared in a manner analogous to that described for polystyrene. But especially with the nitrocellulose some further precautions against degradation had to be taken. The nitrate was stored in methanol and dried over night in vacuum before the preparation of the solutions which were then stored in the dark.

B. The measurements of viscosity

1. Cleaning of the viscometer

After the measurement of a polymer fraction, the viscometer was first rinsed repeatedly with a good solvent for the polymer. The solvent had to work for many hours and in that way the adsorbed polymer was removed to a large extent. Then, eventually via a second solvent completely soluble in both the solvent first used and water, the viscometer was rinsed with water. For the following cleaning of the viscometer we usually used a mixture of sulfuric, hydrochloric and nitric acids in water. The mixing was made in the viscometer in order to obtain an evolution of heat. This acid mixture

was usually employed for twenty-four hours and then the viscometer was rinsed repeatedly with water and with ethanol, which was left for at least two hours. Finally it was washed repeatedly with the solvent which was to be used in the following measurements. All the liquid was filtered into the viscometer through a filter G4. Such cleaning was only made when the measurements of a complete fraction were finished, or under special circumstances.

2. *Filling of the viscometer*

The measurements of a polymer fraction were made in such a manner that the efflux time of the viscometer for pure solvent was first determined and then the efflux times for the different solutions in order of increasing concentration. Due to the above the cleaning was completed by carefully rinsing with the solvent, which was to be used in the following measurements. Then, as the volume of liquid in the viscometer might fluctuate within relatively wide limits, it was not necessary to dry the viscometer after the cleaning, but it could be allowed to immediately filter the solvent into the same. A special sintered-glass filter funnel, which was connected to the opening of the viscometer with a ground (glass) joint, was used. The liquid was pressed through the filter by means of a diminutive excess of pressure. Then the viscometer was closed according to chapter 3 (p. 404). As an additional guarantee for waterproof closing a layer of picein lacquer was put on at the ends of the rubber tube. When changing from solvent to solution and to still more concentrated solutions, the original liquid was first carefully emptied, the viscometer was rinsed twice with 15–20 ml of the solution to be used at the next measurement, and then finally it was filled with the solution. The part of the liquid that was impossible to pour out from the viscometer did not exceed $\frac{1}{2}$ ml, and as the filling volume was not below 50 ml, the concentration error due to this dilution was surely not high enough to influence the results obtained even in the most extreme case. Thanks to this repeated rinsing with solution some other advantages were gained. In this way the filter funnel was rinsed with solution so that it was possible for the filter to adsorb high polymer molecules to a rather high extent before the part of the solution to be used in the measurement passed through the filter. In this way its concentration was surely reduced but little due to adsorption in the filter. In the same way it was possible to get an adsorption layer on the walls of the viscometer (probably not complete) before the final solution was poured in.

3. *Measurements*

After the viscometer was filled with liquid it was placed in the thermostat. A thermostating time of about half an hour was always sufficient for a constant temperature to be attained. But it was found that it was necessary to wait about one hour until the adsorbed layer on the walls of the viscometer was almost in equilibrium with the solution. For that reason the viscometer was always held in the inverted position for at least one hour after that the "upper" bulb (4, Figs. 2 and 3, pp. 403, 404) and the capillary were filled and possible air bubbles in the capillary removed by means of a temporary forcible tilting. The suspended level viscometer was then ready for measurement. The Ostwald viscometer on the other hand was first placed in a horizontal position and the volume to be used in the measurement adjusted as earlier described (p. 402). After this adjusting it was held in that position

for a further ten minutes and then it was put in position for measurement. In the following measurements the Ostwald viscometer was held in the inverted position for 15 minutes and for 10 minutes in the horizontal position. These times were chosen to have the drainage as small as possible (p. 410).

The measurement of the efflux time was made as previously described (p. 407).

PART IV

RESULTS, DISCUSSION AND CONCLUSIONS

Chapter 8. Results from measurements with the highest precision obtainable

From the experience with some preliminary measurements it was obvious that the middle fraction of polystyrene in toluene would give the solutions that were most suitable for measurements with the highest precision. For these the suspended level viscometer with three capillaries was chosen. The diagrams obtained from one of these series of measurements are given in Figs. 5 and 6.

Fig. 5 comprises the whole concentration interval, whereas Fig. 6 shows in detail the conditions at very low concentrations only. In addition to the η_{sp}/c values that are corrected also for the adsorption effect, as described in Chapter 4, p. 413, the uncorrected values of η_{sp}/c have been plotted versus c . Primary data for these diagrams are given in Table 5. (It may be observed that the relatively great change of the efflux times for the lowest concentrations does not mean that the true η_{sp}/c is changed by a corresponding amount, as we have a similar change for all the three capillaries.) The slope of the curve gives the value 0.3 for k' in Huggins' equation (p. 399).

When going to discuss the diagrams we must first emphasize that for this polymer-solvent system the "critical concentration", according to Boyer and Spencer (13), that means the concentration corresponding to the hexagonal close-packing of spheres with diameters two times these obtained for the random coils of the molecules

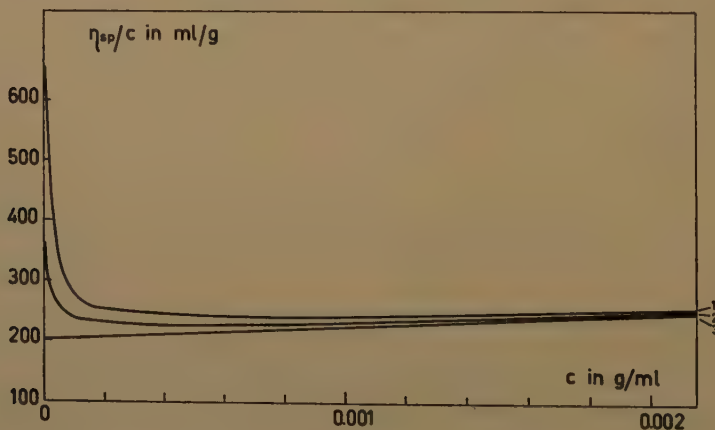


Fig. 5. Viscosity of polystyrene in toluene. True $\eta_{sp}/c = f(c)$, 3, and uncorrected $\eta_{sp}/c = f(c)$, 1, 2. (The curve from S2 is omitted.)

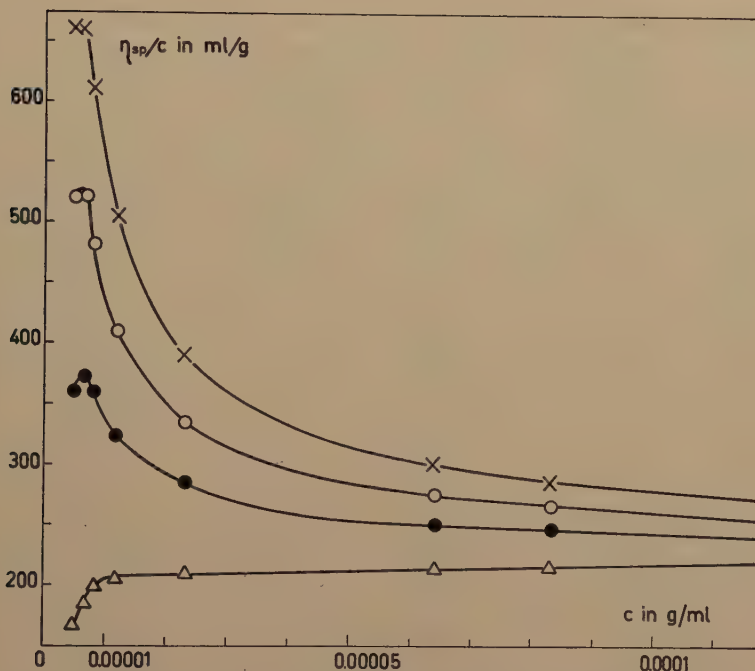


Fig. 6. Viscosity of polystyrene in toluene. True $\eta_{sp}/c = f(c)$, Δ . Uncorrected $\eta_{sp}/c = f(c)$: $\bullet$, for $d = 0.5$ mm; $\circ$, for $d = 0.3$ mm; $\times$, for $d = 0.2$ mm.

in question in the actual solvent was estimated to 0.0003 g/ml. Then we choose for our discussion an interval of concentration from 0.00001 g/ml to 0.002 g/ml or from a concentration 30 times lower to a concentration 7 times higher than the calculated "critical" one. In spite of the fact that we thus surely have the "critical concentration" in the interval investigated, there are no greater deviations from the linear curve obtained by plotting the true η_{sp}/c vs. c . It is evident from the curves and the primary data that the accuracy is very high in this interval.

A critical examination of the curve will but show that there is a very small "upward convexity" already at concentrations of about one third of the "critical" one. But still at concentrations thirty times lower than this the deviation from the above straight line is not greater than that which would correspond to a decrease of η_{sp}/c of two per cent. If the change in slope at these concentrations much lower than the "critical" one, is real, it would mean a certain experimental confirmation of the theories of Simha (1) and of Riseman and Ullman (2). But we must observe that the correction to be applied to the uncorrected η_{sp}/c value obtained with the S1 viscometer is very great at concentrations beneath 0.0001 g/ml and an error of only a few per cent of that correction is enough to change the slope of the curve with several hundred per cent. For this reason it is impossible to decide if there is a change in slope of the true viscosity curve.

For the middle fraction of polystyrene in toluene here investigated it is first at concentrations 50–100 times lower than the "critical" one as accidental errors become

Table 5. Efflux times of solutions of polystyrene fraction No. 12.

Concentration g/ml	Efflux times in s		
	S1	S2	S3
0	881.52	1532.80	1112.14
	51	83	12
	48	80	13
	51	80	10
	51	81	12
	53	81	09
0.00000470	882.89	1536.52	1115.50
	99	59	60
	883.07	69	69
0.00000631	883.54	1537.70	1116.64
	55	75	67
	62	82	76
0.00000761	883.93	1538.40	1117.27
	93	48	25
0.0000118	884.88	1540.10	1118.69
	91	15	75
0.0000232	887.29	1544.64	1122.21
	31	65	23
0.0000567	893.98	1556.57	1131.06
	97	64	09
0.0000732	897.36	1562.60	1135.54
	37	57	53
0.000145	911.72	1587.74	1153.99
	71	74	1154.00
0.000230	928.61	1617.37	1175.62
	63	37	63
0.000552	995.15	—	1260.43
	12	1733.65	44
0.000865	1060.35	1847.40	1342.84
	33	38	90
0.00134	1166.02	2031.81	1477.52
	1165.99	85	48
0.00196	1315.50	2292.90	1667.40
	49	88	38

noticeable. The difficulties encountered are formidable here. The time difference for solution and solvent is not greater than 0.1 per cent of the efflux time. This means that a relative error of 0.01 per cent in efflux time will give an error of ten per cent in η_{sp}/c . Though outside the scope of this work it may be of some interest to discuss the viscosity curve at these concentrations below the concentration interval now discussed. In Fig. 6 there is an indication that the curve might "bend down" here at the lowest concentrations. A similar "bending down" has been observed many times (see also Fig. 7). But with the high corrections here due to adsorption it is very difficult to decide definitely if the "bending down" is real. The correction which must be made to the value of η_{sp}/c obtained from S1 (see p. 413) is in any case several times the difference between the true η_{sp}/c and the value that corresponds to an extra-

polation of the linear curve. It is also possible that the assumption for the correction that the effective layer is of the same thickness for all capillaries is not quite valid here where the adsorption is very small. Much can be said in favour of a "bending down" of the curve. Firstly an evident tendency to a "bending down" has been obtained. Under no circumstances is there a "bending up". This effect occurs in spite of the fact that attempts have always been made to prevent as well as possible a "bending down" due to different experimental errors; the shortest conceivable efflux time for the solvent has been used and the dilution method ought to guarantee that the concentration is not diminished, etc. But there are many sources of error which work for a "bending down". One of the most serious is the decrease in concentration due to adsorption and another the reduction of the viscosity due to absorption of water. Others are degradation of the polymer molecules and higher adsorption in the finer capillaries than in the wide one (the solution is often in contact with these finer capillaries for a longer time than with the wider one), so that the corrections are too high.

A fact that may be noted is that in nearly all the measurements "the bending down" of the extrapolated curve appears at a concentration where the curves which are not corrected for adsorption have a maximum. Leaving pure errors of measurement out of the question, this maximum must be caused by one or both of the following reasons: (1) The adsorption isotherm bends down to quite a large extent at concentrations lower than that of the maximum, so that for these concentrations $da/dc > a/c$, if a is the thickness of the apparent adsorption layer. (2) We have a rapid reduction here in the true value of η_{sp}/c . Much is to be said in favour of a real "bending down" of the true viscosity curve, but there are also indications that sometimes the condition above of the adsorption, $da/dc > a/c$, may be fulfilled (see p. 429). A simultaneous appearance of these two effects is perhaps more than a coincidence. If there is indeed a change in the viscometric behaviour at this extreme dilution, as may be indicated by the "bending down" of the extrapolated curve, it is not impossible that there is also another effect of the same nature that may be observed here. The course may be the following. If the viscosity increases rapidly the hydrodynamic action on the adsorbed layer must increase very much (we have the maximum gradient here). Hence it is possible that in that interval of concentration where the adsorbing surface is perhaps not totally covered by polymer molecules, the adsorption is rendered more difficult. On the other hand at higher concentrations with a stronger adsorption this effect may be diminutive. (See also p. 429.) This would mean that the two eventual effects are both hydrodynamic in nature. Finally it must be pointed out again that the intention of the measurements now performed was not to investigate this interval of concentration and thus it is impossible to explain this behaviour with any certainty.

About the possibility to investigate still lower concentrations some brief comments may be made. It seems almost impossible to reach concentrations essentially lower than those now examined, due to the fact that many errors, earlier of minor importance, will now be significant. At the same time many correction terms become so large that the corrected values of η_{sp}/c at best can only be approximate. Even if some of the best improvements are tried it is doubtful if more pertinent information will be obtained. Such improvements include use of wider capillaries, use of more suitable polymer-solvent systems mainly with low adsorption and a series of measurements with decreasing concentration to ensure complete adsorption before the solution to be used in the actual measurement is poured into the viscometer. It must

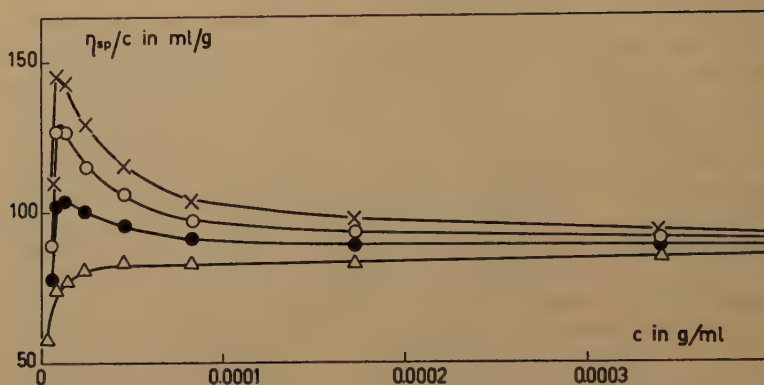


Fig. 7. Viscosity of polyvinyl acetate in toluene. True $\eta_{sp}/c = f(c)$, Δ . Uncorrected $\eta_{sp}/c = f(c)$: $\bullet$, for $d = 0.5$ mm; $\circ$ for $d = 0.3$ mm; $\times$ for $d = 0.2$ mm.

also be remembered that all these improvements will greatly increase the time for a series of measurements which is at present about one month.

As the results obtained here are in many respects quite different from those given in the most interesting recent paper, published by Tuijnman and Hermans (8), a comparison between their data and ours will now be made. They examined polyvinyl acetate dissolved in toluene. Among other things, the dependence of the gradient on viscosity was studied. They believe that their measurements have shown that the anomalous viscosity curves are primarily due to non-Newtonian effects. This is not confirmed by our measurements. We have also made a few measurements with polyvinyl acetate-toluene solutions though primarily for investigations of other problems than those here mentioned (see p. 432).

The polyvinyl acetate used was kindly placed at our disposal by Dr. F. W. Peaker, Chemistry Department, University of Birmingham, Birmingham, England. The samples we have received are two of the fractions he described at the Macromolecular Symposium in Prague, 1957 (31); a branched fraction under the code number SM in reference 31 and a linear fraction used as "backbone" in the preparation of the branched one. Their data are given in Table 6.

For this first comparison with the results of Tuijnman and Hermans only the measurements with the linear fraction are of any interest.

From Fig. 7 it is clear that the diagram of this polyvinyl acetate fraction is essentially equal to that of polystyrene. The extrapolated curve is linear down to concentra-

Table 6. Fractions of polyvinyl acetate.

Sample No.	Frequency of branching in number of branches per 1000 monomer units of "backbone"	$[\eta]$ (tetrahydrofuran) in 100 ml/g	Huggins' constant k'
SM	2.14	1.790	0.53
SM "backbone"	—	1.335	0.33

tions much lower than the lowest one examined by Tuijnman and Hermans. The thickness of the adsorbed layer in our viscometer is about 500 Å at a concentration while they report a value tenfold higher. Tuijnman and Hermans have also determined the adsorption directly and then they obtained the very high value of about 0.01 mg/cm². This means that they have a polymer concentration of approximately 0.2 g/cm³ in the adsorbed layer. Unfortunately no results of adsorption of polyvinyl acetate on glass, besides these mentioned, seem to have been published. But results for other similar polymers such as polystyrene, polymethyl methacrylate and polyvinyl chloride have been published by Jenckel and Rumbach (32) and Fendler, Rohleder and Stuart (33). The values given in these papers are only about 0.0015 and 0.0003 mg/cm² respectively. Even if these two values are very different from each other they are definitely lower than the result of Tuijnman and Hermans. A thickness of 5000 Å would further necessitate a layer at least 5 molecules thick even if they are not deformed at all. This value seems rather high. Thus the values of Tuijnman and Hermans for the adsorption are perhaps too high. But then it is necessary to ask if the curve (see Fig. 4 in ref. (8)), on whose shape this calculated thickness is somewhat dependent, is correct. If we assume a more reasonable value for the thickness, the S-shape of the curve will probably be essentially less accentuated. This would also mean that the gradient dependence is essentially smaller than stated. The S-shaped curve seems moreover to mean that an important part of the total decrease in viscosity with increase of the shearing stress (zero to infinity) occurred over a very

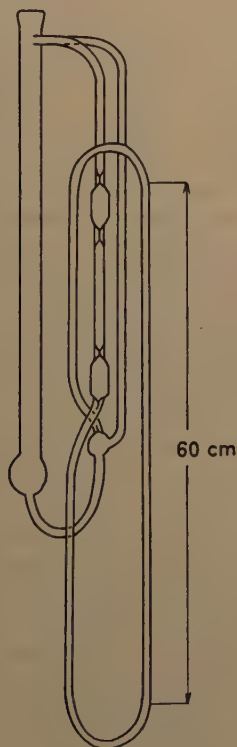


Fig. 8. Viscometer for two different gradients.

limited interval, i.e. $0.4 - 1.6 \text{ g cm}^{-1} \text{ s}^{-2}$. This is in opposition to all experience of this Institute and for this reason we have made an effort to determine if our polyvinyl acetate behaves in a similar manner. We have not aspired to make a systematic study of the gradient dependence, as Tuijnman and Hermans did, but to examine a solution with two different gradients which are equivalent to the highest and lowest values that the above authors used. From such a study it is possible to obtain the total gradient dependence of the viscosity in the interval in question. The dependence found by Tuijnman and Hermans was about 1 % of the viscosity. Such a great change of viscosity is very easy to observe. But efforts have to be made to ensure that it is a *gradient dependence* and *no other effect* which is determined. A modified Ubbelohde viscometer has been constructed and is shown in Fig. 8. It has two bulbs to permit measurements to be made at two different gradients. A list of its dimensions and constants is given below:

Capillary length, 150 cm
Capillary diameter, 0.03 cm
Mean hydrostatic head, 30 and 7 cm
Max. shearing stress (toluene), 1.3 and $0.3 \text{ g cm}^{-1} \text{ s}^{-2}$
Efflux volume, 3 and 3 ml
Efflux time (toluene), 5000 and 21,000 s

Measurements have been made at the concentration 0.0009103 g/ml . The relative viscosities obtained are 1.0787 and 1.0790 for the highest respectively lowest gradient. It is clear from the results that, within the limits of error, no gradient dependence may be observed. A critical examination of the possible errors that may be serious in these measurements shows that both drainage and surface tension errors surely are greater in the measurements of Tuijnman and Hermans than in ours. They use a much smaller bulb, lower mean hydrostatic head, and must have an external pressure for higher gradients. Thus it seems certain that the gradient dependence of our solution is not higher than about one tenth of the value they reported. Of course it must be emphasized that the molecular weight of our sample is lower than that of the fraction used by Tuijnman and Hermans. The intrinsic viscosities are 80 ml/g and $130\text{--}140 \text{ ml/g}$ respectively. But this difference alone cannot explain the very different results. In any case we may surely conclude that in the system polyvinyl acetate-toluene now examined the anomalous viscosity concentration curves are due only to adsorption and not to non-Newtonian effects.

It may be pointed out here that with the thickness of the adsorbed layer given by Tuijnman and Hermans, the relative reduction of the volume of their very small viscometer bulb is great enough to explain the fact that their viscosity curve on the whole has a very marked "upward convexity". This behaviour has never been observed in any of our measurements.

Summing up what has been said here it is evident that: (1) All the anomalies that have been observed in this actual concentration interval around the "critical concentration" are only due to the fact that no correction of adsorption effects have been applied. (2) The true viscosity curve is perfectly linear through this interval of concentration but at concentrations much lower than the "critical concentration" there is some evidence of a "bending down" of the curve.

Chapter 9. A brief presentation of the measurements with different fractions of polystyrene and nitrocellulose

Figs. 9–13 show the diagrams η_{sp}/c vs. c obtained from measurements with polystyrene in toluene and nitrocellulose in ethyl acetate. The diagrams in Fig. 9 are from measurements with the suspended level viscometer.

From Figs. 9–11 it is obvious that for the different polystyrene fractions we always have the usual type of “viscosity curves” when no corrections for the adsorp-

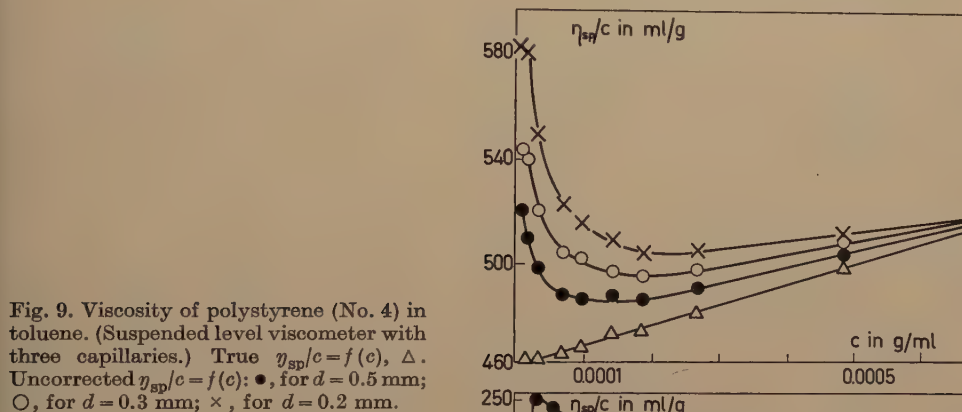


Fig. 9. Viscosity of polystyrene (No. 4) in toluene. (Suspended level viscometer with three capillaries.) True $\eta_{sp}/c = f(c)$, Δ . Uncorrected $\eta_{sp}/c = f(c)$: $\bullet$, for $d = 0.5$ mm; $\circ$, for $d = 0.3$ mm; $\times$, for $d = 0.2$ mm.

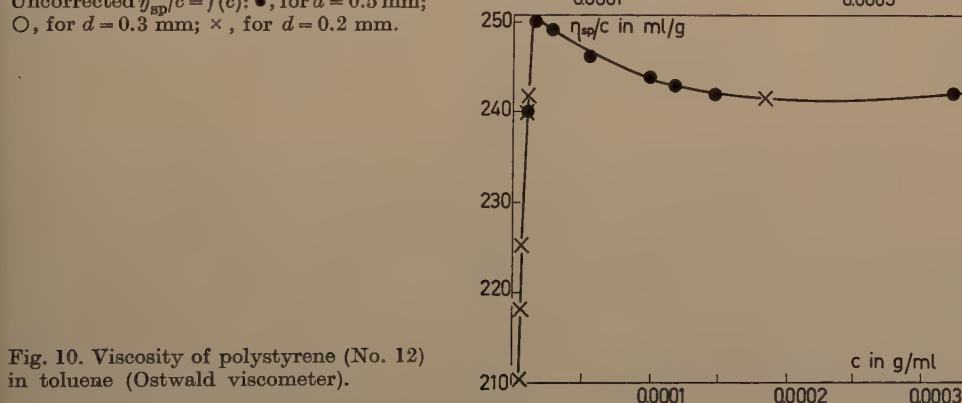


Fig. 10. Viscosity of polystyrene (No. 12) in toluene (Ostwald viscometer).

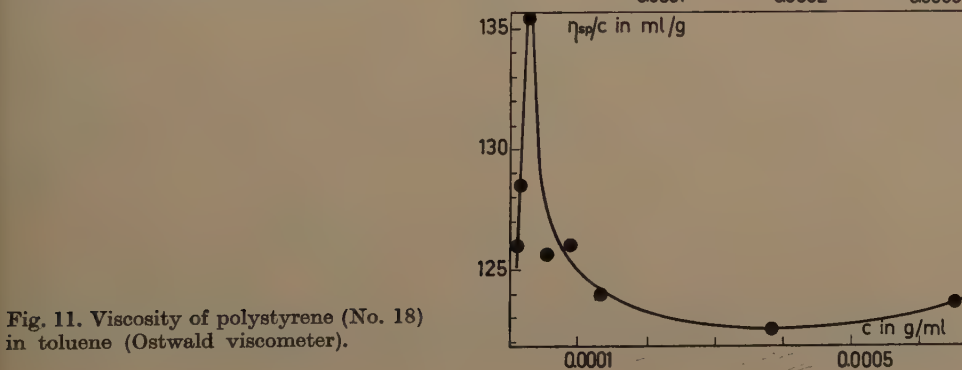


Fig. 11. Viscosity of polystyrene (No. 18) in toluene (Ostwald viscometer).

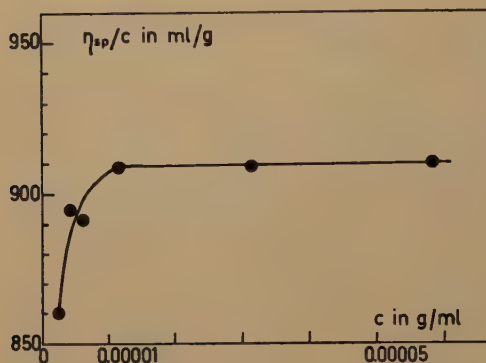


Fig. 12. Viscosity of nitrocellulose (No. 2) in ethyl acetate (Ostwald viscometer).

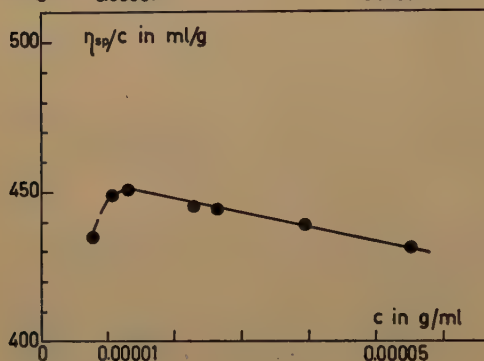


Fig. 13. Viscosity of nitrocellulose (No. 6) in ethyl acetate (Ostwald viscometer).

tion effect are applied. The concentrations of the obtained minima are at least qualitatively in agreement with the conditions of Eq. (4) in reference 12 which gives the concentration dependence of the minimum, assuming that it is due to an adsorption. It may also be observed that the magnitude of the radius of curvature changes with the molecular weight qualitatively as Eq. (5) in the same reference states. The very abruptly "bending down" of the curves of fractions Nos. 12 and 18 may be observed. For fraction No. 12 the lowest concentrations have been reinvestigated with solutions obtained from a new stock solution. The two series agree very well.

The most interesting fact of the nitrocellulose diagrams in Figs. 12 and 13 is that the maxima of the curves are not as marked as those of the polystyrene curves. This may be due to a smaller thickness of the adsorbed layer of the rodshaped nitrocellulose molecules than of the coiled polystyrene molecules; about the same explanation as may be given for the different results of Batzer (19) for linear and branched molecules (see p. 432). Higher degradation of the nitrocellulose may be another explanation.

Chapter 10. Adsorption isotherm, obtained from viscosity measurements

As has been mentioned in one of the preliminary reports (11) it is possible to determine the thickness a from the uncorrected η_{sp}/c values obtained with the different capillaries. We wish to discuss the diagram a vs. c , i.e. the adsorption isotherm that

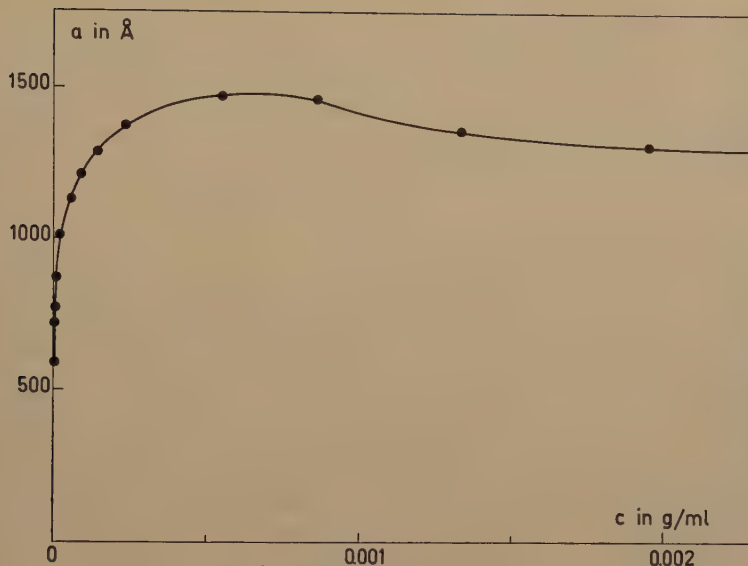


Fig. 14. Adsorption isotherm for polystyrene (No. 12) in toluene. $a = f(c)$.

corresponds to the viscosity curves of Figs. 5 and 6. Fig. 14 gives this adsorption isotherm.

We see that the magnitude of a corresponds to an adsorbed layer of the order of one or two molecular diameters in thickness. This is of about the same order as given in the preliminary report. An examination in detail of several adsorption isotherms has shown that at the lowest concentrations there is a small indication that $da/dc > a/c$. This might explain the maximum of the uncorrected viscosity curve. The reasons for this rapid reduction of the adsorption with concentration may be that at these extremely low concentrations when the adsorbing surface has not yet been totally covered with adsorbed molecules, the hydrodynamic action on the adsorbed molecules is relatively high and thus the total adsorption diminishes further. (See also p. 423.) In reference (11) it was indicated that there was a tendency for a to decrease with an increased concentration, when a concentration of 0.001 g/ml was reached. This seems to be verified now. A possible explanation of this reduction in a is that the molecules are not so expanded when the concentration increases. The maximum of the isotherm is obtained at the concentration where the effective volume of the newly adsorbed polymer balances the compression. It may be of some interest to compare the experimental compression curve with that theoretically estimated.

The concentration dependence of the expansion factor α is determined theoretically by Kawai (34) and Krigbaum (35). The formula derived by the latter is

$$\alpha^5 - \alpha^3 = \frac{C_M M^{\frac{1}{2}} (1/v_1 - 2\chi_{12})}{1 + x v_2 (1/v_1 - 2\chi_{12})} \left\{ 1 - \frac{2 C_M M^{\frac{1}{2}} v_2 / \alpha^3}{1 + x v_2 (1/v_1 - 2\chi_{12})} + \dots \right\} \quad (10)$$

Here C_M is a constant

$$C_M = (27/2^{\frac{1}{2}} \pi^{\frac{1}{2}}) (\bar{v}^2 / N V_1) (M / \bar{r}_0^2)^{\frac{1}{2}};$$

$\bar{v}$ is the partial specific volume of the polymer, N the Avogadro number, V_1 the molar volume of the solvent, $\bar{r}_0^2$ the unperturbed mean-square displacement between chain ends, x the ratio of the molar volumes of polymer and solvent, v_1 and v_2 the volume fractions of the solvent and the polymer, and χ_{12} the interaction parameter characteristic for a given polymer-solvent pair at a given temperature. According to Kawai and Saito this may be approximated (36) to

$$\alpha^3 = 1 + (\alpha_0^3 - 1) / \{1 + (1 - 2\chi_{12})x\bar{v}c\}, \quad (11)$$

where α_0 is the value of α at $c=0$.

We calculate the concentration dependence of a linear molecular dimension that has the same magnitude as a at the concentration 0.001 g/ml (we very approximately estimate the dimension of the adsorbed molecule to be the same as that of the molecule in solution). With $\alpha_0^3 = 3.10$ and $(1 - 2\chi_{12})x\bar{v} = 1600$, which values are approximately valid for polystyrene in toluene ($\bar{M}_v = 800,000$), we obtain the dotted line in Fig. 15, whereas the full line is the adsorption isotherm as given in Fig. 14. As the concentration $c_1 = 0.001$ g/ml is almost fifty per cent higher than the concentration of the maximum, it may perhaps be assumed that the adsorption has reached saturation at the concentration interval examined, so that the adsorption isotherm really gives the compression of the molecules. If we compare the values at two concentrations we have:

$$c_1 = 0.001 \text{ g/ml}, \quad a_1 = 1420 \text{ Å}, \quad \alpha_1^3 = 1.81$$

$$c_2 = 0.002 \text{ g/ml}, \quad a_2 = 1310 \text{ Å}, \quad \alpha_2^3 = 1.50$$

$$a_1/a_2 = 1.08, \quad \alpha_1/\alpha_2 = 1.06$$

Although the agreement between the two curves is quite good,¹ it is impossible from this comparison alone to obtain any further information about these phenomena. This would need a careful estimation of the concentration of the adsorbed layer and a theoretical calculation of the change of its thickness with concentration. On the other hand if such measurements may be assumed as adequate for the determination of the expansion of molecules it is easy to perform some improvements of the experimental methodics.

It may be pointed out that to be able to calculate the value of a with any accuracy at these relatively high concentrations the Eq. (2) does not approximate the "kinetic energy" correction with the desired accuracy. Schurz (37) has given the correct expression of the correction,

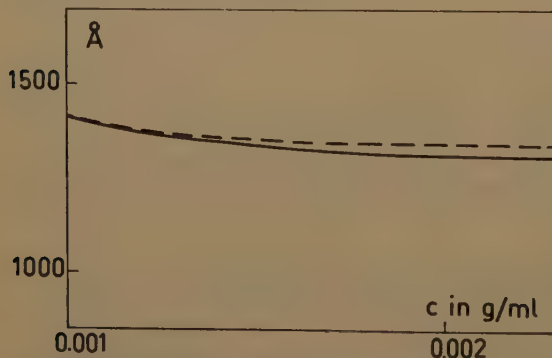


Fig. 15. Adsorption isotherm at higher concentrations (—), and the concentration dependence of the expansion factor (-----). $a = f(c)$ and $\text{const.} \times \alpha = f(c)$.

¹ Note added in proof.—A better approximation than that of Kawai and Saito will give a still better agreement between the two curves.

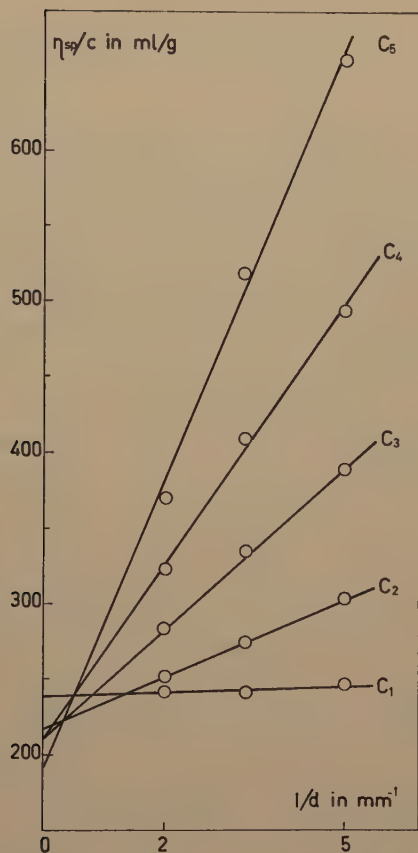


Fig. 16. Diameter dependence of uncorrected η_{sp}/c values. $c_1 = 0.00134$, $c_2 = 0.0000567$, $c_3 = 0.0000232$, $c_4 = 0.0000118$ and $c_5 = 0.00000631$ g/ml.

$$\eta_{sp} = \{\eta_{sp}\} \left(1 + \frac{t t_0^2 + B t_0}{t t_0^2 - B t} \right). \quad (12)$$

But this is here approximated to

$$\eta_{sp} = \{\eta_{sp}\} \left(1 + B \frac{t_0 + t}{t t_0^2} \right) \quad (13)$$

which may be used at the highest concentrations if the value of B/t_0^2 is not exceptionally high (the approximation is indeed better at higher concentrations).

Finally Fig. 16 shows some diagrams, used for the calculation of a , where the η_{sp}/c values are plotted versus the reciprocal of the diameter of the capillary, $1/d$. The graphs are practically straight lines down to the lowest concentration where a slight "upward convexity" may be observed. Hence we have quite good experimental consistency with the claims of the hypothesis (p. 413), that the diameter of the capillary is diminished a little due to adsorption.

Chapter 11. A comparison between the present results and some other recent data

The anomalies of the curves of Takeda and Turuta (4) seem to be adsorption effects (38). The same is surely true for the curves of Streeter and Boyer (5) and Umstätter (6). Boyer and Streeter expressed the opinion that the adsorption can explain neither the molecular weight dependence of the minimum of the curve nor the time dependence of the viscosity they found (39). But the present author (12) and also Takeda and Endo (38) have later shown that the molecular weight dependence is really to be expected. With reference to the time effect, it seems as though the time dependence of the viscosity is only one factor which effects the efflux time, and thus the apparent viscosity when the solution is stored in contact with the capillary. Umstätter declares in a recent paper (40) that he has convincing evidence, both experimental and theoretical, that the anomalies of viscosity are not caused by an adsorption effect. But since a treatment of this evidence, which is directly related to Umstätter's paper (41), has been published by the present author the latter will not be discussed here. (See also a paper by Boyer (42), especially the discussion.)

With reference to Batzer (19) who states that only branched but not linear molecules give a minimum-maximum in the viscosity curve, the present author has earlier (12) suggested the following explanation. An adsorbed monomolecular layer of spherical molecules probably has a greater thickness than a corresponding layer of linear molecules if we have about the same molecular weight for both types of molecules. Some experiments have been made with the suspended level viscometer (some of the earlier experiments were made with the double viscometer described in reference (11)) to see if this explanation is valid, but as yet no definite answer can be given. But some interesting observations were made in connection with these experiments and will be described here. Two different types of polymers have been investigated: (1) Polymethyl methacrylate, linear, and a branched copolymer of methyl methacrylate and glycol dimethacrylate. These polymers were prepared in collaboration with Dr. A. F. V. Eriksson at this Institute. (2) Polyvinyl acetate, linear and branched. These samples were, as already mentioned, kindly placed at our disposal by Dr. Peaker. The properties of the polymers are given in Table 6.

The measurements with polymethyl methacrylate and the corresponding copolymer seemed at first to verify Batzer's results very well. But then we became suspicious that the copolymer might contain traces of crosslinked polymer gel particles. After 30 minutes of centrifuging in a Spinco model centrifuge at 25,000 r.p.m. the marked difference between the two polymers also disappeared. That only extremely small quantities of gels were necessary to cause this effect is clear from the fact that no visible depositions were to be found in the tubes after the centrifuging, and there were no other signs that the solutions were not true ones. The dissipation of the marked difference between the two fractions does not mean that the above hypothesis about these phenomena is wrong. A copolymer of this kind can only have a few branches per molecule which was also confirmed by sedimentation measurements.

The polyvinyl acetates prepared by Peaker seemed to us to be suitable for an investigation of this type. It is true that Peaker had observed signs that the branched polymers with the highest branching frequency were not totally soluble, but this was not the case for the fraction we received. From our work with the branched polymer

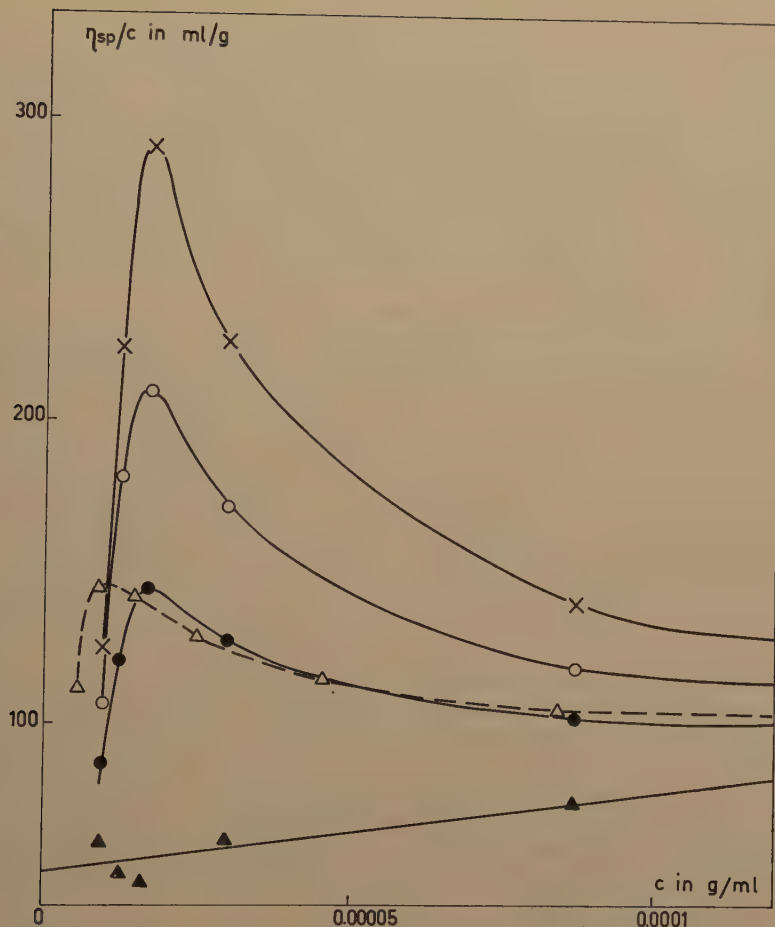


Fig. 17. Viscosity curves for branched polyvinyl acetate. True $\eta_{sp}/c = f(c)$, $\blacktriangle$. Uncorrected $\eta_{sp}/c = f(c)$: $\bullet$, for $d = 0.5$ mm; $\circ$, for $d = 0.3$ mm; $\times$, for $d = 0.2$ mm. (----, linear polyvinyl acetate; obtained with the S3 viscometer; $d = 0.2$ mm.)

we think, however, that it is clear from various observations that also here a cross-linking unfortunately had occurred. Nevertheless we used it for some measurements. Before these were undertaken, we first centrifuged the stock solution at about 3000 r.p.m. (rotor radius ~ 10 cm) for 30 minutes, and then we filtered it twice through G4 filters. Figure 17 gives the viscosity curves for this branched fraction. The dotted line is the corresponding curve of the linear fraction obtained with the S3 viscometer, i.e. the same curve as the highest one in Fig. 7. There is a much more accentuated maximum for the branched fraction than for the linear one. But it is of course doubtful if this effect is due to branched molecules or gel particles. The thickness of the adsorbed layer is greater than that of the linear fraction, this is true, but it is not unreasonably great. With a value of $1200 \text{ \AA}$ at the concentration 0.0001 g/ml it is still much smaller than that given by Tuijnman and Hermans

($\sim 5000 \text{ \AA}$) (8). While centrifuging the solution in a Spinco centrifuge at 25,000 r.p.m. for 30 minutes, there were possibly some very weak traces of gels.

All this shows very distinctly, however, that we must proceed with the greatest caution when working with branched molecules prepared according to methods where cross-linking is not totally avoided. For instance it seems very probable that the different peculiar viscosity effects obtained by Peaker with that branched polymer may be explained by the presence of gel particles. (See also next paragraph.)

In several other papers it is stated that the viscosity anomalies of the type minimum and eventual maximum in the η_{sp}/c vs. c curve cannot be explained as an adsorption effect. Kapadia for instance asserts this opinion. He found anomalies of this type for some different rubber solutions (43). Later he stated (44), that direct adsorption experiments with glass wool showed that practically no adsorption took place. The constancy of the concentration was determined viscometrically. He also reported that some preliminary observations indicated that an uncoiling effect in the dilution caused the anomalies obtained. Unfortunately very few details have been given, and for this reason it is unprofitable to discuss this work. The same may be said about a short summary by Kapur and Gundiah (45), where the high viscosity at low concentrations is stated to "be attributed to the extension and disentangling of polymer chains". Nevertheless some general comments may perhaps be permitted. Kapadia mentions (reference (43), p. 196) that two of the solutions of rubber were not easily filtered through a No. 4 sintered glass disc. This may of course indicate that gel particles were present, and it has already been pointed out that those cause very marked maxima in the viscosity curve. It may also be mentioned here in connection to the uncoiling hypothesis that Batzer's results with branched and linear polymers seem to be inconsistent with the same. An uncoiling hypothesis ought to demand that the linear molecules are more sensitive to dilution than the branched ones. Experiments with linear and branched polyelectrolytes would give a corresponding, amplified difference. Any really suitable measurements for such a comparison do not seem to have been made, but the work of Schaefgen and Trivisonno (46) with linear and multichain polyamides of the same chemical composition may indicate that for polyelectrolytes the uncoiling of linear molecules affects the viscosity more than that of branched molecules.

Very recently a paper has been published by Hirai (47), where he examines thoroughly most of the papers on viscometric anomalies and explains theoretically such anomalies as pure viscosity effects. But for the theoretical interpretation he assumes that the concentration of the minimum is a "critical" one and, as it has now been shown that this assumption is incorrect, this evidence probably does not have any value.

Kawai and Saito (36) on the other hand modified Huggins' equation by means of an expression, Eq. (11), for the concentration dependence of the expansion factor α . In that way they deduce an equation that at least qualitatively describes the minimum of the viscosity curves obtained. The dependence of the shape of the curve and of the position of the minimum on the molecular weight is quite adequate. An examination of their course of action shows that they first give evidence that k' in Huggins' equation

$$\eta_{sp}/c = [\eta] + k'[\eta]^2 c \quad (14)$$

is not a real constant. Then they deduce a new expression for k' but also substitute for the constant term $[\eta]$ an expression due to Flory

$$[\eta] = K M^{\frac{1}{2}} \alpha^3. \quad (15)$$

Finally they substitute α with an expression in which the concentration dependence of α is included. *They cannot state the reason for this change of the constant term $[\eta]$.* This is important since it is the expansion of this term that actually causes the "bending up" of their theoretical curve. The contribution of the other term to η_{sp}/c is only a few per cent in the interval of low concentrations, and in addition this term decreases with decreasing concentration. Further it may be mentioned that unreasonable high values of α are demanded to explain the increase of η_{sp}/c by several hundred per cent, which sometimes has been obtained in certain experiments.

SUMMARY

The purpose of this work has been to study the viscometric behaviour of extremely dilute solutions of high polymers. Especially the concentration interval around the so-called "critical concentration" has been examined in detail.

1. Equipment for the very precise determination of viscosity is described. Details of special interest are:

(a) Ostwald viscometer; totally enclosed during measurements; the liquid comes to the upper viscometer bulb when the whole viscometer is tilted; the volume of working liquid redetermined at each run; special arrangements to avoid errors due to drainage.

(b) Suspended level viscometer; three different viscometers in one with different diameters of capillary to be able to correct for the adsorption error under No. 2a below; totally enclosed during measurements; the liquid comes to the upper viscometer bulbs when the whole viscometer is tilted.

(c) Thermostat with proportional control of temperature.

(d) Photographic method to measure efflux time.

2. In the discussion of error especially two errors have been treated:

(a) Adsorption error due to the fact that adsorption diminishes the diameter of the capillary.

(b) Kinetic energy error. A modified method for the determination of the kinetic energy correction has been submitted.

3. Curves of η_{sp}/c vs. c have been obtained using different capillary diameters and clearly show that *the usual anomalies of viscosity curves at very low concentrations are only due to adsorption effects.*

4. Curves of η_{sp}/c vs. c corrected for adsorption effects are perfectly linear down through the "critical concentration" interval.

5. Certain indications have been observed that there is a "bending down" of the viscosity curve at concentrations *much lower* than the calculated "critical concentration".

6. It has been shown that the great gradient dependence, pointed out by Tuijnman and Hermans, does not occur.

7. Effects similar to those of Batzer with branched and linear polymers have been obtained. These effects are due to adsorption, but it could not be decided definitely whether the adsorption layer of the branched polymer contains traces of gel particles and thus is more accentuated.

8. Some recent theoretical calculations that the usual anomalous viscosity curves in reality are true ones, are shown not to be convincing.

9. Adsorption isotherms giving a (= the thickness of the adsorbed layer) vs. c have been obtained from viscosity values.

10. The curve of a vs. c has a maximum at a rather high concentration. This may be due to a compression of the molecules with increasing concentration. A comparison is given between this curve and a theoretical curve taking account of the concentration dependence of the expansion factor α .

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X-ray orientation investigations on some Swedish cellulose materials

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With 11 figures in the text

Because of their importance cellulose materials (treated or untreated) of different kind and origin are probably the most extensively investigated of all the natural materials. Orientation investigations by X-rays have been made by many authors, a few examples of which are given in the references (1–15).

As the properties of wood, being a biological material, probably vary with climatic and other conditions at the place of growth, the aim of the experiments reported in this paper was mainly to investigate the orientation properties of some Swedish wood specimens. X-ray investigations on Swedish materials have been made (e.g. (16)) but with a somewhat different aim. Because of the variations, which may be expected in a biological material, the present experiments must be considered only as preliminary, as a complete investigation of the influence of the different factors which might affect the orientation properties probably would require a very large number of experiments with specimens well defined with respect to their growth conditions.

In addition to the experiments with wood a few experiments with two kinds of grasses are also given in this paper.

Experimental

The method applied was the same as that described in a previous paper (17), i.e. X-ray diagrams were taken with thin wood specimens placed normal to the primary beam. The radiation scattered was recorded on a plane film which was also placed normal to the primary beam. The interference circles in the diagrams were probably due only to the cellulose constituent of the specimens, at least it was not possible to see that other constituents, which no doubt were present, had influenced the position of the interference circles. From the diagrams relative values for the orientation were calculated from microphotometric blackening recordings along the strongest interference circle corresponding to the plane (002) in the cellulose crystallites. An X-ray diagram and the corresponding microphotometer recording is shown in Fig. 1. Ni-filtered Cu-radiation from a Philips' X-ray tube, fed by half-wave rectified tension of 40 kV and 20 mA, was used. The pinhole system had a diameter of 1 mm, the distance from specimen to film was 30 mm and the exposure time 75 minutes.

The wood specimens were prepared as follows. A disc about 2 cm thick was sawn out normal to the trunk of the tree investigated. From the disc strips about 5 mm wide were cut out radially

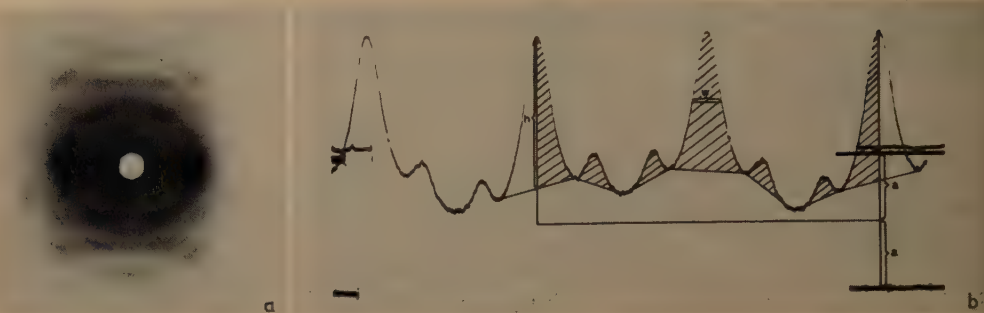


Fig. 1. X-ray diagram and the corresponding microphotogram to explain the measurements.

(a) The X-ray diagram; spruce 2, radius I, annual ring 22, spring wood, specimen vertical.

(b) The microphotogram. For this and for the following microphotometer curves the peaks correspond to blackening maxima. The traces of the recordings of the blackening averages on both sides of the main interference circle are seen at both ends of the figure (cf. (17)). The estimated background under the main interference circle is drawn in the middle between these traces.

On the main microphotometer curve a full turn of 360° is marked by vertical lines. A line (the "boundary line") is drawn through the minimum points of the main curve. The quotient between the hatched area and the area (for 360°) between the main curve and the background is the orientation quotient. The height, h , of a peak and the orientation angle, w , are measured as shown in the figure.

and in these strips specimens were cut from different annual rings. If possible the specimens were taken so as to contain only one of the two kinds of wood, spring wood (light) or summer wood (dark), which usually are clearly distinguishable in an annual ring. The specimens mostly had a cross-section of about 0.8 mm (less in some wood specimens from thin annual rings). The grass specimens were taken as strips about 0.8 mm broad from the wall of the straw.

The wood specimens were dried for about 6 hours at 102°C , treated in an extraction apparatus with a mixture of equal parts benzene and 96 % ethyl-alcohol for 6 hours and washed in warm water. Afterwards the specimens were boiled with 1 % KOH solution under a reflux condenser for 1 hour and again washed with warm water. After treatment with 1 % HAc solution and washing, the specimens were finally dried at 102°C for 6 hours. This treatment is about the same as the first steps in the treatment used by Nilakantan (10). This author also made a chlorination process which, according to his results, slightly decreased the parallel-orientation in his specimens but otherwise had little influence upon the X-ray diagrams. The extraction with benzene and alcohol removed resins and fatty substances and this process in some cases gave clearer diagrams. The treatment with KOH solution removed part of the hemicellulose but the effect of this latter extraction was hardly visible on the X-ray diagrams (cf. (10)). It is of course possible that the alkali treatment also caused a slight degradation of the cellulose molecules.

The number of grass specimens was small and a systematic treatment was not applied.

Remarks concerning the method

On the microphotograms the width of the main peak at half the maximum height was measured. For some diagrams also the integral width, i.e. the area of the peak divided by its height, was calculated. These two values for the width usually agreed fairly well (Fig. 8b) and only the former will be considered in the discussion. Further the area between the microphotometer curve and an

empirical baseline (cf. (17)) was divided into two parts by a broken line (the "boundary line") through the minimum points on the curve. The quotient between the upper part of the area under the microphotometer curve and the total area was calculated and taken as a measure of the amount of oriented material in the specimen. The measurements are illustrated in Fig. 1.

The angles related to the widths of the peaks on the microphotometer curves and the quotients between the area of the peaks and the total area under the microphotometer curves are called orientation angles and orientation quotients respectively, and the terms orientation values or orientation parameters are used for both the angles and the quotients.

The measurements were made directly on the microphotometer curves, i.e. on blackening distribution curves where the blackening was measured as the light absorbed in the X-ray films, as described in a previous paper (17).

The specimens with a normal cross-section of 0.8 mm contained hundreds of cells, about 200 with a cross-section of the tracheids of 0.05 mm, and thus the values measured represent an average for this number of cells. As the tracheids do not end in certain horizontal planes in the annual rings there must be contributions both from the middle parts of the cells and from their ends.

A characteristic feature of the wood cells is the layering of the walls (e.g. (18)). The main part of the material and practically all the cellulose in fibrillar form is present in the secondary wall which is generally built up of three layers, S_1 , S_2 , and S_3 in order from the outside of the wall (notation according to (19)). The number of layers may be larger or smaller; e.g. in spruce usually only two layers, S_1 and S_2 , are observed (20). The layer S_2 is generally thicker than S_1 but probably the relative amounts of material in the two layers vary. The layers differ from each other in the direction of the cellulose fibrils in relation to the length of the cell. For normal wood the fibrils in S_1 and S_3 have almost a transverse direction in relation to the cell axis, whereas the fibrils in S_2 form a steep spiral around it. In compression wood (e.g. in spruce) the spiral in S_2 is less steep than in normal wood (e.g. (18)).

The layered structure of the cell wall may influence the intensity distribution in the interference circle considered in different ways; some of the possibilities for the normal wood are discussed in the following. It is assumed that the crystallites are oriented rotationally symmetric with regard to the whole cell.

One possible case is that the crystallites in the layer S_1 are oriented in such a way that the plane (002) (giving the strongest interference circle) is mainly parallel to the fibre axis in which case both S_1 and S_2 always contribute to the maxima on the equator of the X-ray diagrams. A second possibility is that the crystallites in the layer S_1 are rotationally oriented in the fibrils, in which case they would give an interference circle with the highest intensity at the meridian of the diagram. The maximum intensity should depend upon the amount of scattering material in S_1 and upon the degree of alignment of the fibrils in this layer. However, in this case it is seen that the intensity at the meridian is lower than in an equatorial arc scattered from the same amount of material, having the same alignment of the fibrils, but with these oriented parallel with the cell axis instead of transverse to it. Another possible arrangement is that, in S_1 , the plane considered is oriented normal to the cell axis (i.e. orientation of higher order in S_1 as in the first case) when an arc might or might not (depending upon the degree of orientation) be obtained at the meridian of the diagram. For the last two cases it should be characteristic that the intensity of the meridional arc increases if the zone of reflection is brought up to the cell axis (or to one of the poles of the "reference sphere", cf. (17, 21)) either by decreasing the wavelength or by tilting the specimen in such a way that its axis (parallel to the axes of the tracheids) forms an angle equal to $(\frac{1}{2}\pi - \theta)$ (θ is the glancing angle) with the primary beam. The increase of the intensity should be largest for the latter case mentioned. Still other cases are possible, e.g. if the spiral of the fibrils in S_1 is not flat the meridional arc may show two maxima (cf. (15), p. 127, and (18)).

Assuming values for the amounts of material in the two layers and distribution functions for

the crystallite orientations, more quantitative estimations of the intensity distributions could be obtained (cf. (21)) but at present such calculations probably would not be of much use.

From the preceding discussion some points concerning the orientation values, measured for the normal wood, may be obtained. Assuming the layer S_2 to contain considerably more material than S_1 , the shape of the microphotometer curve is mainly determined by the orientation of the cellulose fibrils in the layer S_2 (cf. Preston and Wardrop, references in (18)). Consequently the orientation angle which is related to the shape of the peaks in the microphotometer curve depends most upon S_2 . It should be remembered that the orientation angle involves both the alignment of crystallites within the fibrils and the orientation of the fibrils themselves. If, in the layer S_1 , the plane considered is oriented in such a way that most of the scattered radiation falls within the equatorial maxima, the layer S_1 should hardly influence the boundary line (cf. Fig. 1b) between the peaks and the bottom part of the area under the microphotometer curve. On the other hand if the crystallites in S_1 are oriented so as to give an arc at the meridian of the interference circle, it is seen that the boundary line just mentioned may be influenced by material in the layer S_1 . Thus the orientation quotient may depend upon (besides of course the material in S_2) both crystallites, which may be considered as randomly oriented, and crystallites oriented along transverse fibrils in the layer S_1 . The measurement of the orientation values on the microphotometer recordings instead of on intensity distribution curves also affects these values but this influence can be estimated from blackening curves and from a discussion of the background determination (cf. (17)), whereas the influence of the relative amounts of scattering material and of the crystallite orientation in the layers S_1 and S_2 respectively at present constitute the main cause of uncertainty in the significance of the orientation values measured.

Materials

The wood specimens were taken from the species *Picea excelsa* (Swedish spruce). One of the trees had grown in Uppland, the other trees (pulp wood) had grown somewhere in the middle parts of Norrland. The exact position of the place of growth and the situation in the trunk of the sections obtained were not known for any of the specimens. One of the spruce trees from Norrland, denoted spruce No. 1, had very thin annual rings varying from a thickness of about 1.5 mm at the center to about

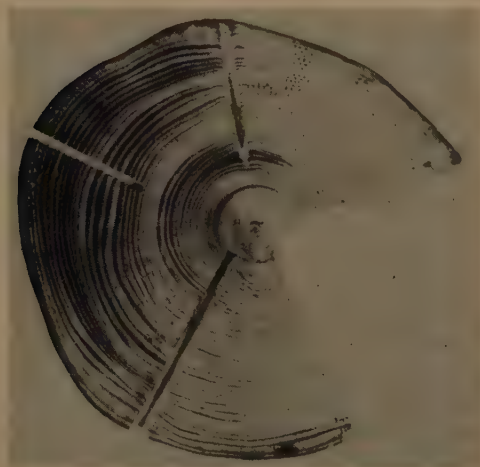


Fig. 2. Cross-section of spruce 2 showing the compression wood section and the radii investigated. Radius I is in the lower left quadrant and radius III is in the compression wood section. The missing part of the cross-section consisted of light soft wood.

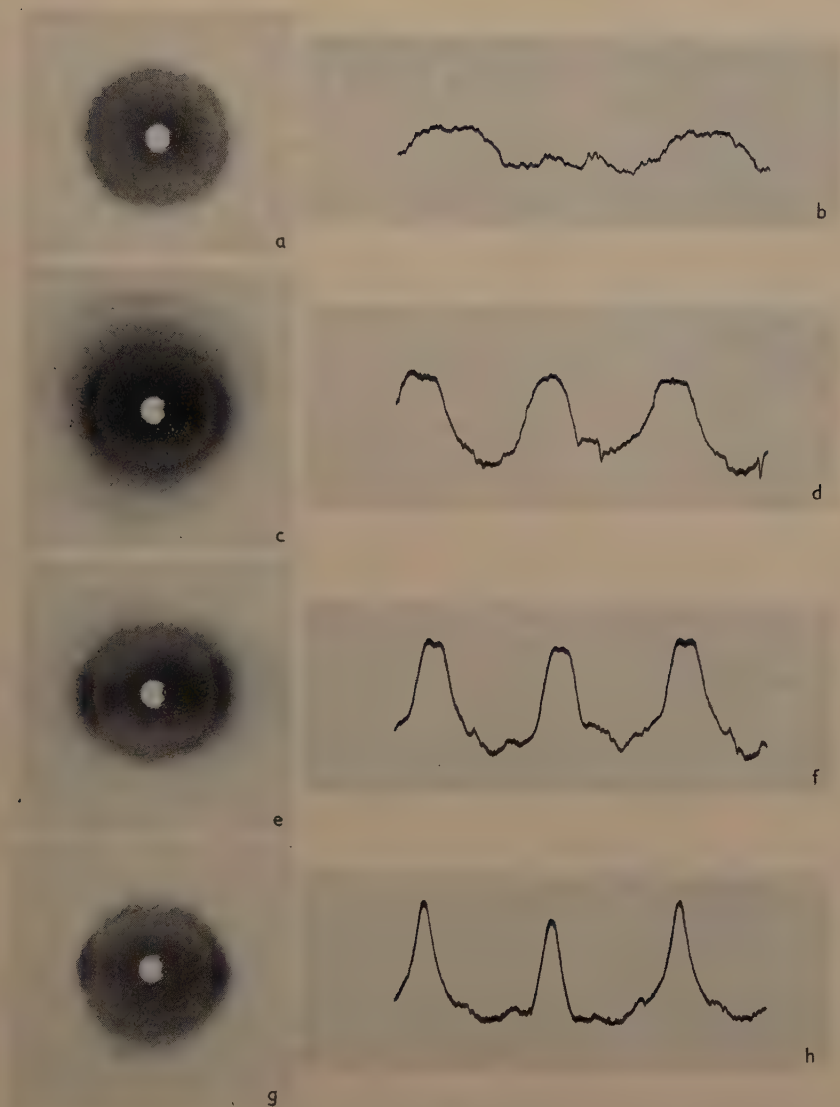


Fig. 3. Some X-ray diagrams and the corresponding microphotometer curves from spruce 1. Only the main curve is shown.

X-ray diagram	Microphotometer curve
(a) Ring No. 1	(b) Ring No. 1
(c) 3	(d) 3
(e) 6	(f) 6
(g) 10	(h) 10

0.2–0.3 mm at the outer part of the trunk. The age of the tree was about 60 years and the diameter of the trunk was about 12 cm. From this tree only the annual rings nearest the center were investigated. The specimen grown in Uppland, spruce No. 2,

was very irregular in its pattern of annual rings. It had a strong section of dark compression wood and its annual rings varied considerably in thickness. The width of one ring varied not only from the dark to the light sections of the trunk but also, for example, within the light section. The section of this tree, which was investigated, had 58 annual rings and its diameter was about 25 cm. A photograph of the cross-section of the trunk is shown in Fig. 2. A third tree, spruce No. 3, was uniform in its ring pattern. The annual rings were about 2 mm broad and the diameter of the bole about 15 cm. From this tree most specimens were taken from the annual rings nearest the pith but some were taken further out at different points of the cross-section.

The grasses investigated were couch-grass, *Triticum repens*, and wheat, *Triticum sativum*, of unknown variety.

The botanic names are from Krok & Almquist, *Svensk Flora*, 22nd ed. Stockholm 1938.

Results and discussion

In the experiments the diagrams were taken with the primary beam normal to the fibre direction and in most cases with the beam both in the tangential and in the radial plane. Only in a few cases with compression wood were differences obtained between the two diagrams of a specimen. An example is shown in Fig. 11 (*g-k*). Unless otherwise mentioned the diagrams discussed here were obtained with the primary beam in the radial plane.

The specimens taken from spruce No. 1 were cut from the springwood in the annual rings nearest the pith. The diagrams (Fig. 3) show a regular increase in parallelism from the pith outwards to about the tenth ring where it tends to become more constant. In some cases it was possible to resolve the peaks into two but only the width at half the maximum height (the orientation angle) was measured. In Fig. 4 the orientation angles are plotted against the number (counted from the pith) of the annual rings.

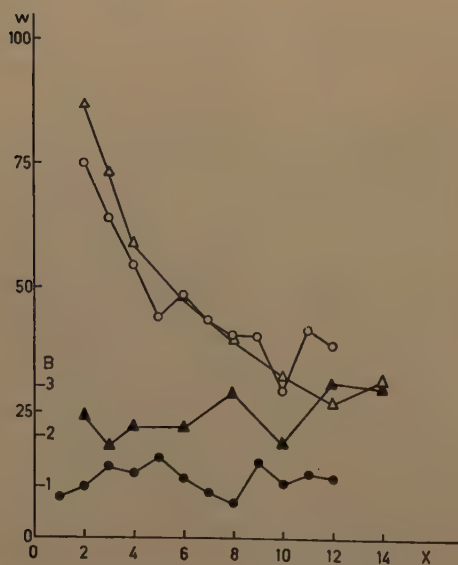


Fig. 4. The orientation angle, w , and the thickness, B (in mm), of the annual rings for spruce 1 and 3, plotted against the number of the rings, X . $\circ$, orientation angle for spruce 1; $\triangle$, orientation angle for spruce 3; $\bullet$, thickness of the annual ring for spruce 1; $\blacktriangle$, thickness of the annual ring for spruce 3.

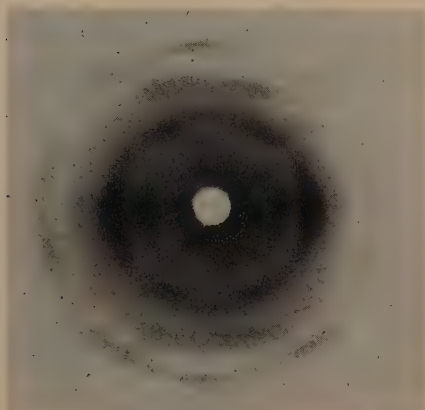


Fig. 5. X-ray diagram of spruce 3, annual ring 14, spring wood.

The diagrams of springwood from the central annual rings of spruce No. 3 gave the same picture as above (Fig. 4). The normal diagrams from spruce No. 3 varied but usually showed a high degree of parallel orientation (Fig. 5).

The increase from the pith outwards of the parallel orientation in the wood (Fig. 4) has been observed and discussed (e.g. (15), p. 160, (18)) and similar effects have also been found for natural materials other than wood (e.g. (22)).

It may also be mentioned here that in the experiments with grass specimens it was found that the parallel orientation in the straw increased with the upward distance from the joint. Fig. 6 shows the orientation angle for some diagrams of a wheat straw plotted against the distance in mm between the lower joint and the exposed part of the specimen which was taken from one of the middle articulations of the straw. The same tendency was also obtained for a straw of couch-grass.

Even within one trunk the orientation differences may be very large as is seen from the diagrams of spruce No. 2 (Figs. 1, 7 and 11). This tree no doubt shows larger orientation differences than usual and is therefore not a good example of the average spruce, but on the other hand because of its abnormality it demonstrates the possibilities of a living tree to respond to different conditions. These conditions may involve the soil (e.g. composition and slope), which should not change very much, but also mechanical factors, depending upon wind exposure and the development of the crown, climatic factors and outer influences of biological origin which might all vary considerably during the lifetime of a tree.

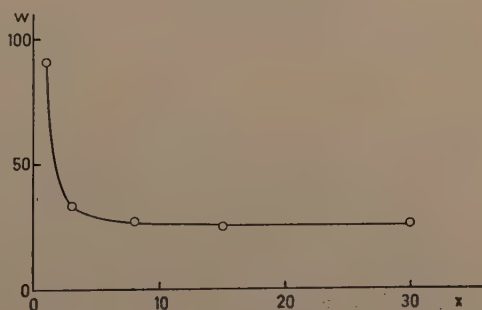


Fig. 6. The orientation angles, w , from exposures of a wheat straw, plotted against the distance, x , in mm from the lower node.

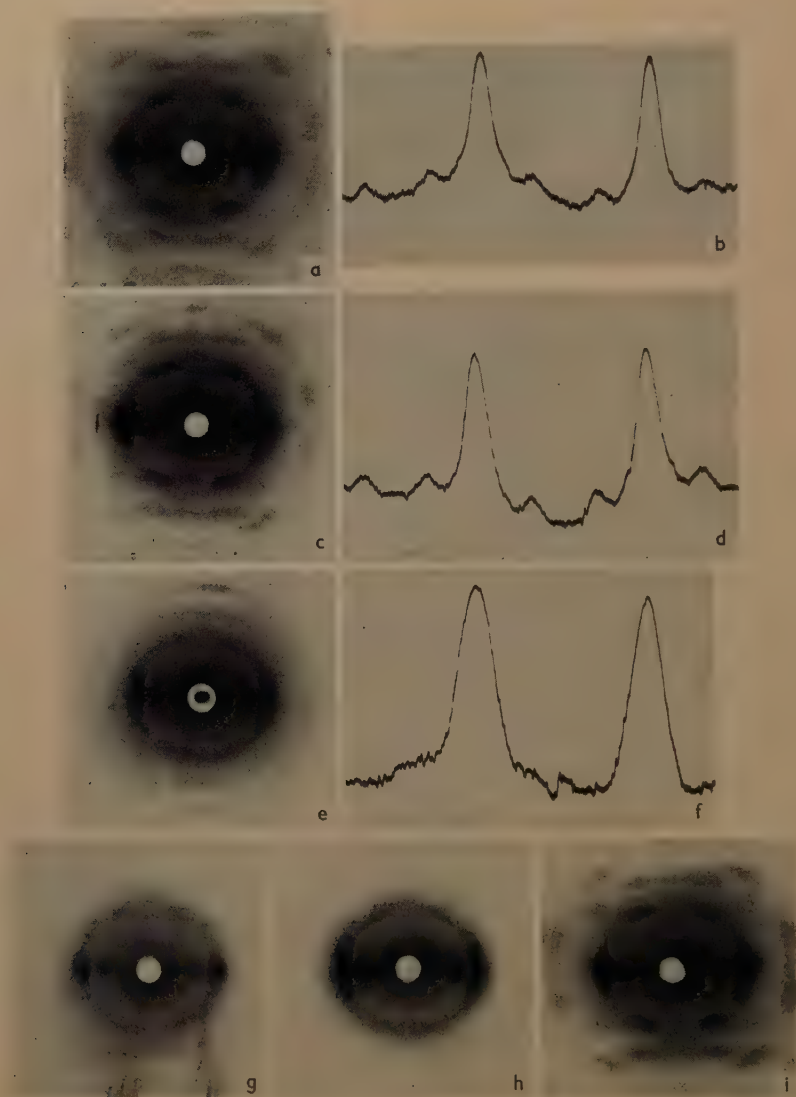


Fig. 7. Some X-ray diagrams and microphotometer curves from spruce 2, radius I.

X-ray diagram	Microphotometer curve
(a) Ring 30, spring wood	(b) Ring 30, spring wood
(c) 30, summer wood	(d) 30, summer wood
(e) 35, spring wood	(f) 35, spring wood
(g) 33, spring wood	(exceptional, see p. 448)
(h) 33, summer wood	
(i) 52, spring wood	

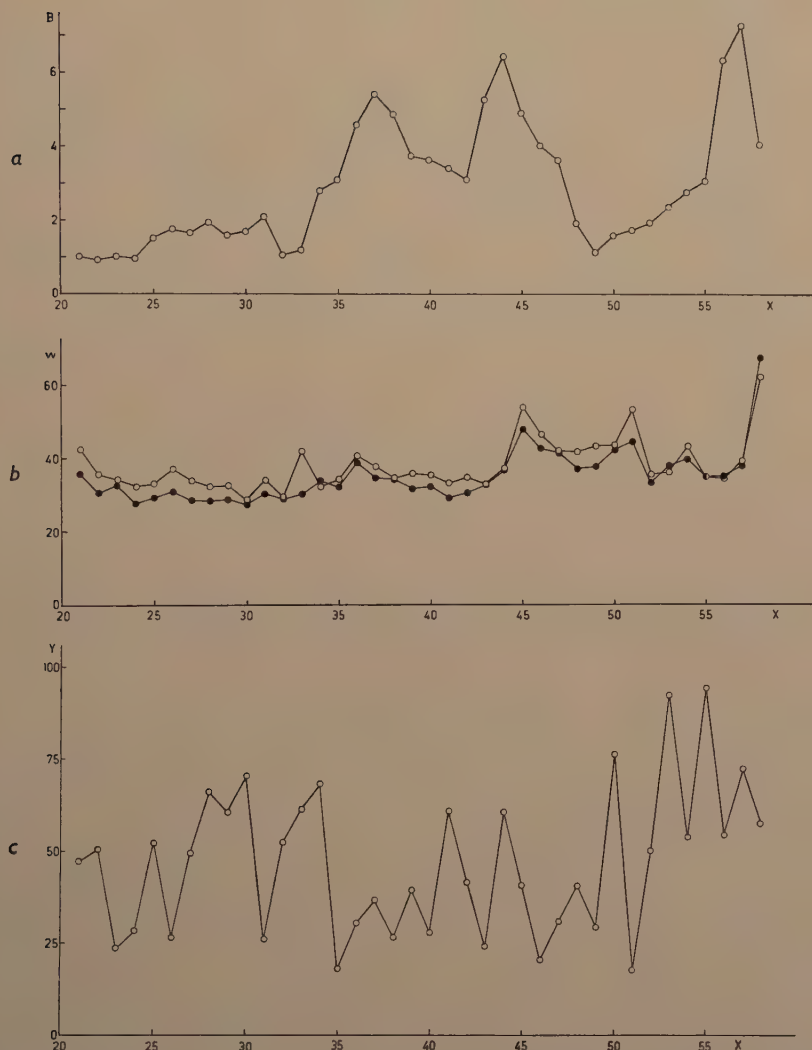


Fig. 8. Measurements from spruce 2, radius I, spring wood, plotted against the number, X , of the annual rings. (a) The thickness, B (in mm), of the annual rings. (b) The width, w , of the peaks: $\bullet$, the width at half the maximum height, the orientation angle; $\circ$, the integral width. (c) The orientation quotient, Y .

Spruce No. 2 was investigated in more detail than the other trees. The specimens were taken mainly along two different radii (approximately radial), which were chosen in the region between the "light" and the "dark" sector in the trunk (radius I) and in the "dark" sector (radius III). A few specimens were also taken along a radius (II) in the light wood but they were not very different to the specimens from radius I.

Some of the diagrams and some microphotometer recordings of the wood in radius I are shown in Figs. 1 and 7. The parallel orientation in these specimens varied consider-

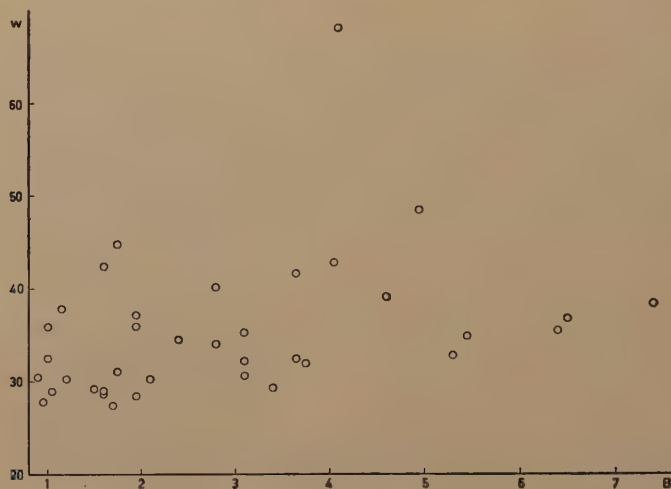


Fig. 9. The orientation angle, w , plotted against the thickness, B , of the annual rings.

ably. The outermost annual ring, number 58 from the pith, was different from the others and gave a diagram which was more diffuse and with a much broader and more flattened maximum.

In Fig. 8 some values measured at different annual rings along the radius I are plotted against the number of the ring counted from the pith. Only the rings from number 21 and outwards were measured because the wood in the central part of the trunk was disturbed by a crack and some knots. Fig. 8a gives the thickness of the annual ring in mm; Fig. 8b gives both the width at half the maximum height of the peak and the integral width, but only the "half maximum" width is considered in the following; Fig. 8c gives the orientation quotient. It is seen from the curves that there are in this case no simple relations between the measured values and the age of the annual ring. It is known that the thickness of the annual rings depends upon the growth conditions and it may be expected that the other values measured also change with these conditions, which, in the present case, were not known in detail. Thus it remains to correlate the measured values.

The orientation angles plotted against the thickness of the annual rings (Fig. 9) show a rather irregular distribution, possibly with a slight increase of the angles with increasing thickness of the rings. The orientation quotients (Fig. 8c) are distributed over a large interval. Part of this scattering is probably due to the method which may give errors of about ten units of the Y-scale (cf. (17)), but the total distribution of the values cannot be explained by the experimental error. To obtain a clearer picture of the relation between the breadth of the annual ring and the orientation values, the measured values corresponding to three successive annual rings were averaged and plotted against the middle number, i.e. the average corresponding to 21, 22, and 23 against 22; 22, 23, and 24 against 23 and so on. This procedure tends to eliminate the experimental errors and other irregularities which may be due to differences in the influence exerted by a growth factor upon the breadth of an annual ring and upon its orientation value respectively. The "three-year averages" for the orientation quotient and the thickness of the annual ring are plotted against the corresponding number in

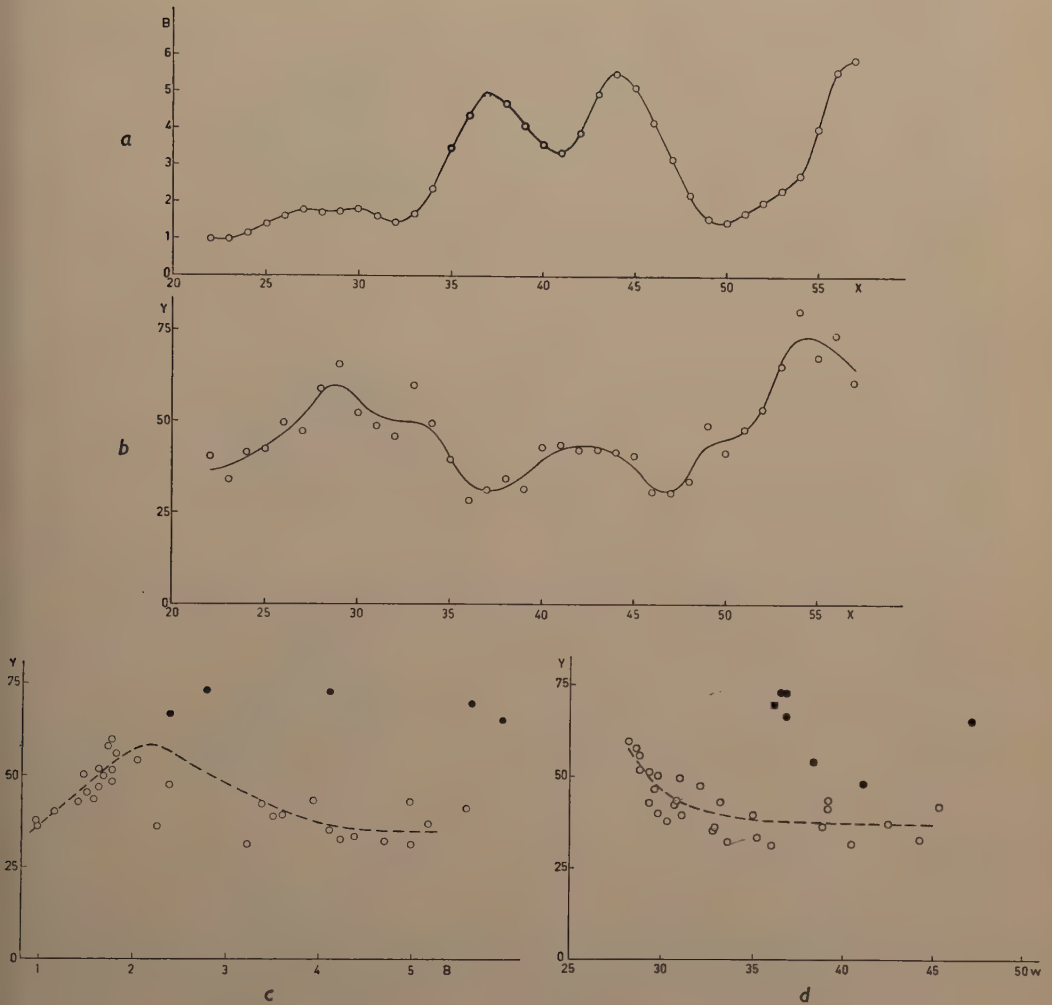


Fig. 10. The "three-year averages" from spruce 2, radius I, spring wood. (a) The average thickness, B , of the annual rings plotted against the ring number average, X . (b) The average orientation quotient, Y , plotted against the ring number average, X . (c) The average orientation quotient, Y (smoothed value from (b)), plotted against the average ring thickness, B . The values corresponding to the seven outermost rings are marked with filled circles. (d) The average orientation quotient, Y (as in (c)), plotted against the average orientation angle, w . The values corresponding to the nine outermost rings are marked with filled circles.

Fig. 10a and b. It is seen that the averaging procedure does not change appreciably the shape of the curve obtained by connecting the widths of the annual rings plotted against the number of the rings. The orientation quotients taken from the smooth curve in Fig. 10b are plotted in Fig. 10c against the corresponding averages of the annual ring breadths. According to this plot the orientation quotient should have a maximum for an annual ring thickness of approximately 2 mm and decrease for higher

and lower values. The tendency is marked by the dashed line. The same picture is obtained also with the calculated average of the orientation quotients but the scattering of the points is somewhat larger. The outermost annual rings do not seem to fit into the general picture. From Fig. 8c it is seen that the orientation values for these rings are more irregular than for the other rings and in Fig. 10c the five highest points, four of which deviate markedly from the other points, correspond to the five outermost averages. The averages of the orientation angles were also calculated. These values plotted against the orientation quotient averages show an increasing tendency for the orientation quotient with decreasing angle (Fig. 10d). In this case the seven points in the upper part of the diagram which deviate from the distribution of the other points correspond to the outermost annual rings. The deviations of the values corresponding to the outermost annual rings from the other values may be due to different causes, either they may be due to the position in the trunk or to external effects. A difference between the outermost annual rings and the others has been found in connection with other investigations (23).

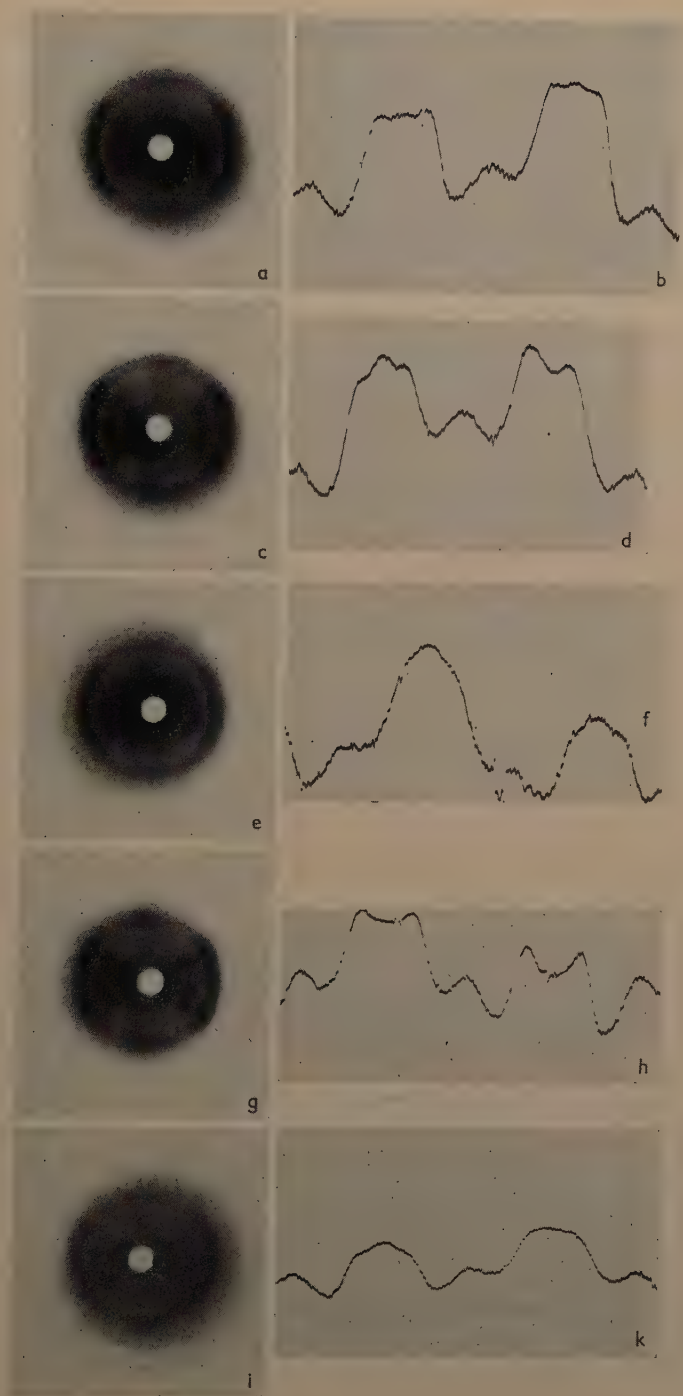
In radius I the rings of summer wood were usually thin and it was possible to get only a few specimens from these parts of the annual rings. Two diagrams of the summer wood from radius I are shown in Fig. 7. The diagrams were usually sharper than the corresponding diagrams from spring wood. However it was not always found that the summer wood gave sharper diagrams than the spring wood (cf. Fig. 7g and h).

The radius III in the compression wood section in spruce 2 was not investigated in as much detail as radius I but in most cases diagrams were taken from both kinds of wood in an annual ring. Some exposures and microphotometer recordings are shown in Fig. 11. It is seen that these diagrams do not show the same parallel orientation as the diagrams for radius I. Usually the diagrams of the summer wood clearly indicate a spiral orientation whereas the diagrams of spring wood are somewhat more diffuse and it might be difficult to determine the type of orientation directly from the X-ray diagrams.

Most of the diagrams which have a broad arc at the equator also have a weak maximum on the meridian which may be due to crystallites in transversely oriented fibrils, or to a partial overlapping at the meridian of the strong arcs at the equator (cf. (15, 18)). Also the small maxima (plane (021)) on the microphotometer curve which correspond to the second layer line, may influence the maximum on the meridian. From the present experiments it is hardly possible to decide with certainty which of the causes is most important in this case. For the compression wood the main maximum of the microphotometer recordings is often somewhat irregular. The summer wood diagrams usually show the two maxima which for a material with spirally oriented crystallites should occur on both sides of the equator, whereas the recordings for the spring wood often have only one broad, and sometimes irregular

Fig. 11. Some X-ray diagrams and microphotometer curves from spruce 2, radius III, compression wood.

X-ray diagram	Microphotometer curve
(a) Ring 49, spring wood	(b) Ring 49, spring wood
(c) 49, summer wood	(d) 49, summer wood
(e) 53, spring wood	(f) 53, spring wood
(g) 53, summer wood, primary beam radial	(h) 53, summer wood, primary beam radial
(i) 53, summer wood, primary beam tangential	(k) 53, summer wood, primary beam tangential



maximum at the equator (cf. (14)). The absence of the minimum on the equator may be due to a less perfect alignment of the crystallites within the spirally oriented fibrils in the spring wood than in the summer wood (which is not likely, cf. (24)), or to a more scattered orientation of the fibrils themselves. This latter case also involves the possibility that the alignment of the fibrils in the layers of the secondary wall does not vary considerably but that there are different layers with different fibril orientation in one cell, or that some of the cells in a specimen have a spiral arrangement of the fibrils and others have the fibrils oriented almost parallel with the cell axis. Again the averaging property of the method makes it difficult to decide between the possibilities even if it does not seem very probable that the different cells within the comparatively confined cross-sectional area of a specimen should have a quite different arrangement of the fibrils. The assumption of layers (or cells) with different orientations might also explain the irregularities of the main maximum on the microphotometer curves. In some cases it seems that on the broad maximum there are three smaller ones, also visible on some of the films. One of the small maxima lies on the equator and the other two on both sides of it (cf. Fig. 11 (*a-d*)). This picture could be obtained by superimposing the diagrams of a spirally oriented specimen and a fibre oriented specimen (cf. (25) on reaction wood), and diffuse spiral and fibre diagrams might give an irregular main maximum.

The general impression of the diagrams obtained may be summarized as follows. For the normal wood the diagrams show a sharper parallel orientation than for compression wood, which often gives diagrams typical for a spiral arrangement of the crystallites. Spring wood and summer wood have diagrams which indicate the same kind of orientation, i.e. fibre type or spiral type. Mostly the denser summer wood gives somewhat clearer diagrams than the spring wood from the same annual ring. All these features agree with the results obtained for other wood materials. The compression wood diagrams usually show an arc at the meridian (also well known) which may be due to different reasons. For the spring wood in the compression section of spruce 2 some irregularities are observed at the equator of the diagrams which may be due to a fibre diagram superimposed on a spiral diagram. Some of the diagrams of normal wood (fibre oriented) are clear and rich in interference spots (Figs. 1, 5, 8, cf. tension wood diagrams, e.g. (25, 26)). Such diagrams, showing a comparatively well developed crystalline structure of the cellulose, were obtained from different trees and it is possible that the Swedish material investigated is, in this respect, different from most other wood materials investigated. In this connection it should also be remembered that the low number of interference spots on the diagrams of the compression wood may be due to the fact that the radiation is scattered over longer arcs of the corresponding interference circles than for the simple fibre diagrams of the normal wood. This weakens the interference spots and they may be undistinguishable.

From the previous discussion it is seen that one of the characteristics of the method applied is that it gives an average for a large number of cells. This is a drawback in so far as the significance of the orientation values which may be obtained from the microphotometer recordings is not quite clear. However, for comparatively sharp parallel orientations (simple fibres or steep spirals), a calculation of the orientation angle and the orientation quotient might be useful as is seen from the graphs (Fig. 10) showing the relations of the two orientation parameters to each other and to the widths of the annual rings. For the spiral oriented materials it would be possible to use the same orientation values although their significance for that case

should be still more uncertain, but it might be better to choose the parameters in a different way.

The property of the method to give an average for the whole specimen irradiated may be utilized to characterize larger pieces of material. For example it is possible to cut out thin radial strips at a given cross-section of a tree and to move such a strip during the exposure in a radial direction across the primary beam. If the speed of the movement is regulated so as to be inversely proportional to the distance from the primary beam to the pith, the resulting diagram will give a type of average of the orientation conditions within a uniform sector of the cross-section and it ought to be possible to estimate an average of the orientation values for the whole cross-section from exposures of a few radii. Such a development of the method would save considerable time and work. Also it should be possible, by using a suitable experimental arrangement with a high effect X-ray tube, to decrease the exposure time by about a factor of ten from the 75 minutes used in the present investigation. If the X-ray orientation parameters for wood could be correlated with the technical properties of the cellulose obtained, orientation determinations then would offer a comparatively fast and simple method to characterize wood of different origins.

SUMMARY

Some specimens of Swedish spruce have been investigated by X-rays with the aim to characterize the orientation properties of the cellulose crystallites in the specimens. From microphotometer recordings of the X-ray fibre diagrams obtained, two different orientation parameters were calculated. One of these, called the orientation angle, was the width at half the maximum height of the main peak of the microphotometer curve. This orientation value has been used by many authors. The other parameter, called the orientation quotient, was calculated from the area under the microphotometer curve and is related to the amount of oriented material. The significance of these orientation values has been discussed.

A few specimens of grass were also investigated with the X-ray method.

The orientation values measured for the annual rings along a certain radius in a tree have been graphically correlated with each other and with the thickness of the annual rings. There seems to be a relation between the orientation quotient and the thickness of the annual ring and between the orientation angle and the orientation quotient.

The general impression obtained from the X-ray diagrams of spruce was that the summer wood in an annual ring gave sharper diagrams, indicating a higher degree of crystalline order than the corresponding springwood ring. Further, the diagrams of normal wood were mostly of fibre type whereas the diagrams of compression wood indicated spiral orientation. The innermost annual rings of normal wood showed indications of spiral orientation; the spiral became steeper in the outward direction and at about the tenth annual ring the usual fibre diagram was obtained. These features agree with the results reported from investigations of other wood materials. A comparatively large number of the diagrams of normal wood were very well defined and in this respect there may be a difference between this and most other cellulose materials.

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Purification of prostatic acid phosphatase

By HANS G. BOMAN

With 9 figures in the text

Introduction

The acid phosphatase of the human prostate is interesting from several aspects. It has been found only in humans and higher apes (1), and it seems to be one of the few known enzymes which may be characteristic for a group of mammals. It is a normal constituent of semen, and Lundquist (2) has shown choline phosphate to be the natural substrate. Gutman and Gutman (3) discovered the appearance of the enzyme in the blood in case of advanced cancer of the prostate, and further references about the pathological significance of the phosphatase can be obtained from the review of King (4). In addition to the interest in the enzyme as such, different preparations have been used for the determination of terminal phosphate groups in structure work on phosphoproteins (5) and nucleotides (see e.g. (6, 7, 8)).

Considering these aspects, rather few attempts have been made to isolate the enzyme. An often used partial purification has been described by Schmidt (6) who also gives references to earlier work. London and Hudson (9) have reported a 4900-fold purification by the use of a laborious procedure which later has been somewhat simplified to give a 3100-fold purification (10). Lundquist *et al.* (11) have obtained a high degree of purification with zone electrophoresis in starch. However, as starting material they used seminal plasma, which is difficult to obtain in sufficient quantities.

The present communication describes a simple method of purification in two steps: The first one consists of an extraction and a dialysis, and the second one is a modification of a chromatographic fractionation described earlier (12, 13). In the previous study (13), lack of material prevented any investigations on the purified enzyme fraction. It has now been possible to examine the product obtained with ultracentrifugation and zone electrophoresis, and to make some additional analyses.

Evidence has previously been presented indicating that samples of prostatic acid phosphatase may contain a phosphodiesterase activity (14). For this reason, prostatic extracts were analysed for deoxyribonuclease (DNAase) activity and found to contain considerable amounts of such an enzyme. A preliminary purification and characterisation of this prostatic DNAase is given in a companion paper (15).

Materials and methods

The *starting material* was prostatic tissue removed from patients at the Surgical Clinic of the University Hospital, Uppsala. It was stored at about -15°C . The amount of phosphatase in the different pieces has varied somewhat from case to

case, and the figures given in the following refer to an average preparation. An attempt to isolate the enzyme from post-mortem material was unsuccessful.

The *protein content* was, as previously described (13), estimated as the extinction at 280 $m\mu$ (E_{280}) using a Beckman DU spectrophotometer. Water was used as a blank when reading fractions from chromatographic or electrophoretic experiments. For concentrated samples which were diluted before reading, the buffer used for the dilution was taken as the blank. The nitrogen determinations were made with the micro-Kjeldahl method of Andersen and Jensen (16).

Dry-weight determinations were made on purified enzyme fractions which had been concentrated by negative-pressure dialysis (17) against 0.1 M citrate buffer of pH 5.0. Equal volumes (0.5–1 ml) of the enzyme solution and the dialysis buffer were freeze-dried simultaneously and the difference between the dry-weight of the enzyme and the buffer sample was taken as the dry-weight of the enzyme material.

The *phosphatase determinations* were a modification of a procedure earlier described in detail (13). The composition of the substrate solution and the determination of the nitrophenol was the same as previously. In the present work, 200 μ l of substrate solution was incubated in an ice-bath with either 2, 5, or 10 μ l of the fraction to be tested. The reaction was stopped after 10–60 sec., as earlier by adding 2 ml of 0.1 N NaOH (13). In case of low phosphatase activities, 10 μ l and 60 sec. was the rule, while for enzyme samples with E_{280} higher than about 0.4, the time and amount of enzyme were reduced. The final values for the activities have been calculated as millimoles of *p*-nitrophenol phosphate split per liter of incubation mixture, during 1 min. at 0° with a volume ratio of 1/21 between the enzyme and substrate solutions. The specific activities have been obtained by dividing the activity with the E_{280} reading for the undiluted enzyme sample.

These conditions give a straight line relation between activity and time as shown in Fig. 1. A plot of the slopes of the lines in Fig. 1 against the volume of added enzyme solution is given in Fig. 2.

The activities obtained with the present method should be multiplied by a factor of 230 to give figures comparable with the data obtained by the method used earlier (13).

The *chromatographic method* was almost the same as described earlier (13), the difference being that the 8% cross-linked resin was substituted with Dowex 50, X-2. With the lower cross-linking the capacity was increased about 4 times. The same result was independently found by Cunningham (personal communication). For a column (72 $\times$ 3.3 cm) with about 600 ml of resin, the bed shrank about 25% during the regeneration (at room temperature) with 2 N NaOH. When equilibrated with McIlvain's citrate-phosphate buffer of pH 5.50 (the starting buffer) the resin returned to its initial volume.

The *ultracentrifugations*. The instrument was a Spinco Model E, and all runs were performed with rotor "Anal A" and a 12 mm cell. In all experiments the solutions had previously been dialysed against 0.1 M Sørensen's citrate buffer of pH 5.0. The base lines were determined in separate experiments with buffer only and the values for the sedimentation constants (s), are corrected to give sedimentation in water at 20°.

The *zone electrophoresis* experiments were performed with the technique developed by Flodin and Porath (18, 19). The dimensions of the column were 107 $\times$ 2 cm and the supporting medium was ethanolyzed cellulose (18). Cooling was achieved with circulating ice-water. The buffer used in the experiment at pH 4.8 (Fig. 6) was made

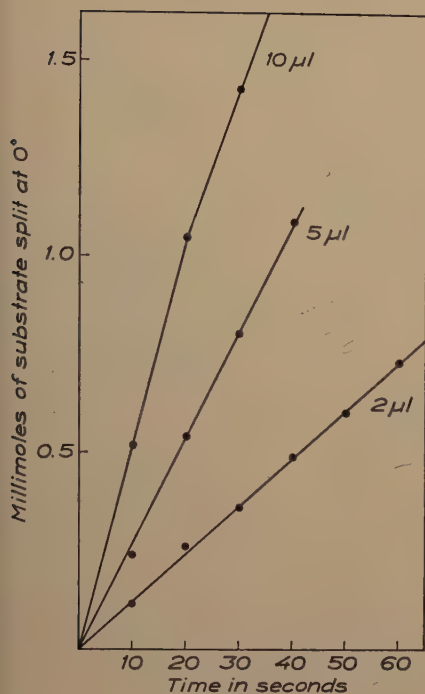


Fig. 1

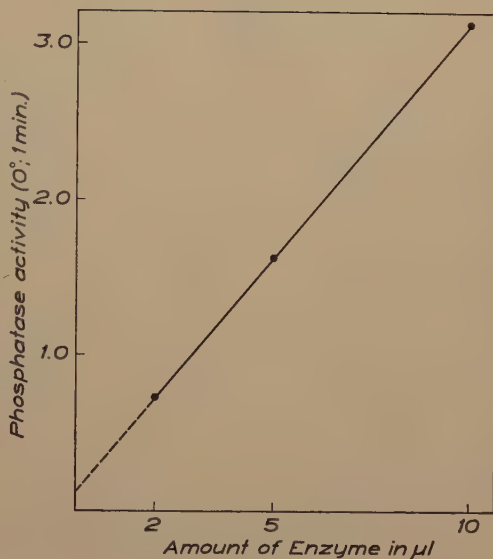


Fig. 2

Fig. 1. The amount of substrate split with increasing time for an enzyme sample with $E_{280} = 1.4$. The substrate solution was 0.02 *M* *p*-nitrophenyl phosphate in 0.1 *M* citrate buffer of pH 5.0, and 200 μ l of this solution was incubated at 0° with 2, 5 and 10 μ l of the enzyme sample.

Fig. 2. Activity with increasing amounts of enzyme. The three points represent the slopes of the lines in Fig. 1. Activity is given as millimoles of substrate split per liter of incubation mixture calculated for a volume ratio of 1/21 for the enzyme and substrate solutions added and 1 min. incubation at 0°.

from 2.24 l of 0.1 *M* citric acid and 1.76 l of 0.2 *M* Na_2HPO_4 , diluted to 20 l. The pH 7.5 buffer (for the experiment in Fig. 7) was made from 0.26 l of 0.1 *M* citric acid and 1.74 l of 0.2 *M* Na_2HPO_4 , diluted to 20 l. The conductivities of the buffers at 0° were equal, $1.3 \times 10^{-3} \text{ ohm}^{-1} \text{ cm}^{-1}$. In both of these experiments, DNP-ethanolamine was used as an indicator when compensating for electroosmosis (20).

The purification procedure

Step. 1. Extraction and dialysis

The frozen prostatic tissue is cut into thin slices (9), weighed, and extracted with 5 times of a 0.01 % solution of Tween 80 in cold distilled water. An efficient extraction is obtained without stirring if the slices are just covered with water, for instance by using a flask with a large bottom surface. (The extraction, as well as the other parts of this preparation, should be performed in a cold room, 4°.) After 24–48 h, the pink opalescent extract is filtered through Pyrex glass wool and then dialysed against distilled water for about three days. The precipitate formed during the dialysis is

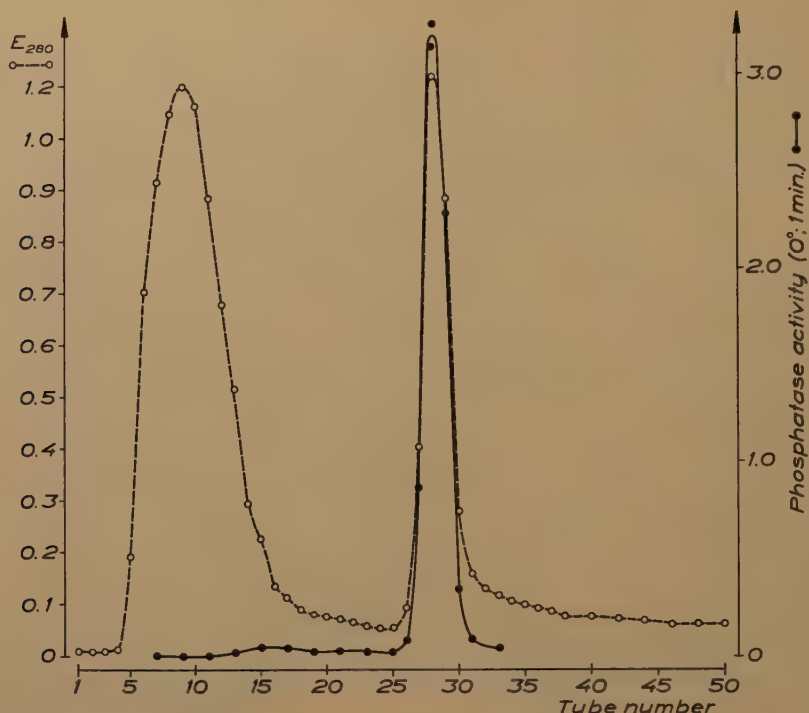


Fig. 3. Chromatographic fractionation of an extract of prostatic tissue (Fraction I) on Dowex 50, X-2. Open circles: Extinction at 280 $m\mu$. Solid circles: Phosphatase activity. Fraction volumes about 6.2 ml.

removed by centrifugation (the precipitate is discarded) and the brownish-pink supernatant is freeze-dried and used for the next step. The dry material obtained (*Fraction I*) has a specific activity of about 0.23, and the amount corresponds to 1–2% of the weight of the prostatic tissue used.

Similar extractions with different buffers have been used earlier (9, 13). These modifications, however, were found to solubilize about 10% more nitrogen and to give lower and less reproducible activity values than obtained with the Tween 80 extraction. Extraction with water only will give almost the same result as the procedure described above, the difference being slightly lower and less reproducible activity figures.

For preparations with a large amount of starting material, a second extraction (similar to the first one), has often been performed. This will result in about 3–5 mg freeze-dried material per gram of starting material.

Step. 2. Chromatography on Dowex 50, X-2

Other modifications of this step have been described earlier (12, 13, 10). The use of *Fraction I* as starting material has, however, permitted a change from gradient to step-wise elution. A typical preparative experiment is carried out in the following way: A column with about 600 ml of pretreated (cf. 13) Dowex 50, X-2 is equilibrated

Table I. Data for different fractions of prostatic acid phosphatase

Fraction	Spec. activity	E_{280} /mg. N ml.	Spot no. in Fig. 8	Comments
I	0.23			
II	2.5—2.8	9—13	1	
II, after rechromatography on Dowex 50	2.49	9.04		
II, after chromatography on DEAE cellulose	2.57	9.9	2	Chromatography in THAM-HCl buffers
II, after zone electrophoresis at pH 4.6	2.50	7.72	3	
II, after zone electrophoresis at pH 5.3	—	12	4	Activity determined by the method in (13)
II, after zone electrophoresis at pH 7.5	2.50	9.2—12		
The phosphatase purified by London <i>et al.</i> (10)	—	19		Assuming a nitrogen concentration of 16 %
The phosphatase purified by Lundquist <i>et al.</i> (11)	—	9.7		Electrophoresis in THAM-HCl buffers

with McIlvaine's citrate-phosphate buffer of pH 5.50 (the starting buffer). Of Fraction I, 600 mg is dissolved in about 30 ml of starting buffer and dialysed against the same buffer for 6–12 h. (Sometimes, a small amount of undissolved material has to be removed by centrifugation after the dialysis.) The solution is then applied to the column (72×3.3 cm) and the effluent, from the time of the start, is collected in a volumetric cylinder. When the solution of Fraction I has passed into the column, elution is first carried out with 55–75 ml of starting buffer. When this volume has passed, a step-wise change is made to citrate-phosphate buffer of pH 6.00. Fraction collection is started after 100–115 ml and about 50 fractions with a volume of 5–7 ml is sufficient. A flow rate of 15–20 ml/h was here obtained with a water pressure of 110 cm. The result of such an experiment is shown in Fig. 3. In this and the following figures, the phosphatase activity is denoted with solid circles and the extinction at 280 $m\mu$ is represented by open circles. The enzyme activity, localized in the second zone (Fig. 3, tubes 27–29), is well separated from the first zone and from the material remaining on the column (13). The tubes from the central part of the second zone are pooled together after checking of the specific activity (cf. Table 1). This solution has been concentrated by negative-pressure dialysis (17) and used for further investigations (Fraction II).

The load here recommended (about 1 mg of Fraction I per ml of equilibrated resin) refers to an average preparation. Occasionally prostatic tissue has been obtained where the enzyme content is considerably increased. Fraction I prepared from such tissue, will with the load used here, give phosphatase activity, in the first zone also. If this represents large amounts of enzyme, it is convenient to collect these fractions for later rechromatography.

The recovery of the activity in Step 2 was of the order 70–80 % and in fraction II a little less than 1 mg dry, dialysable material was obtained from 100 mg of Fraction I.

Investigation of fraction II

Ultracentrifugation

Fraction II has been examined in a Spinco ultracentrifuge, Model E, and Fig. 4 shows three exposures from a representative run. The extinction of the solution at 280 $m\mu$ was 9.43 and it had been dialysed against 0.1 M Sørensen's citrate buffer of pH 5.0. The concentration dependence of the sedimentation constant (s_{20}), was

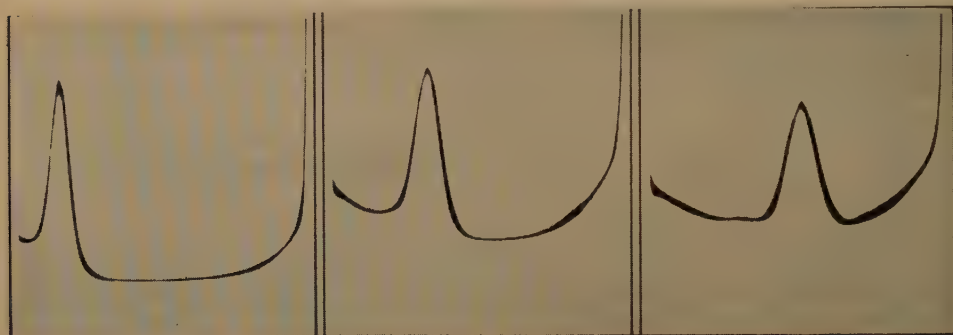


Fig. 4. Ultracentrifugal pattern of prostatic acid phosphatase (tube 28 in Fig. 3), concentrated by negative pressure dialysis against 0.1 citrate buffer of pH 5.0. The first exposure was taken 26 min. after full speed (59,780 r.p.m.), the second one after 58 min. and the third one after 90 min. The E_{280} of the solution was 9.43, and the sedimentation constant obtained was 5.58 S.

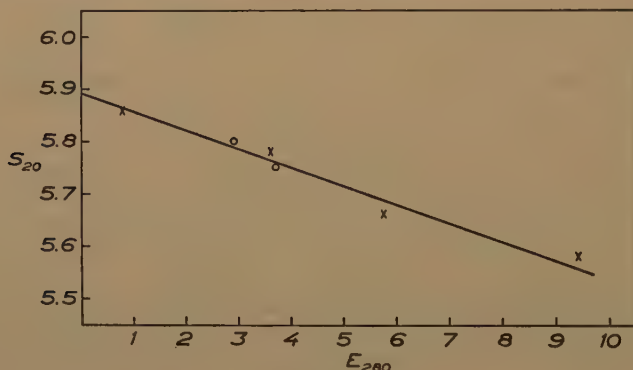


Fig. 5. The concentration dependence of the sedimentation constant for prostatic acid phosphatase. The crosses represent the determination from the experiment showed in Fig. 4 and three dilutions of this sample. The circles stand for the determinations from two different phosphatase preparations which were examined in the ultracentrifuge after chromatography on DEAE cellulose.

determined from three dilutions of this sample (the crosses in Fig. 5) and the sedimentation constant extrapolated to zero concentration, s_{20}^0 , is 5.9 S. In addition, Fig. 5 shows the sedimentation constants for two different samples of Fraction II (the circles in Fig. 5), which were examined after chromatography on DEAE cellulose (21).

Zone electrophoresis

Fraction II has been studied with column electrophoresis (18, 19) at pH 4.8, 5.3 and 7.5 in dilute citrate-phosphate buffers. In the experiment at pH 4.8, the amount

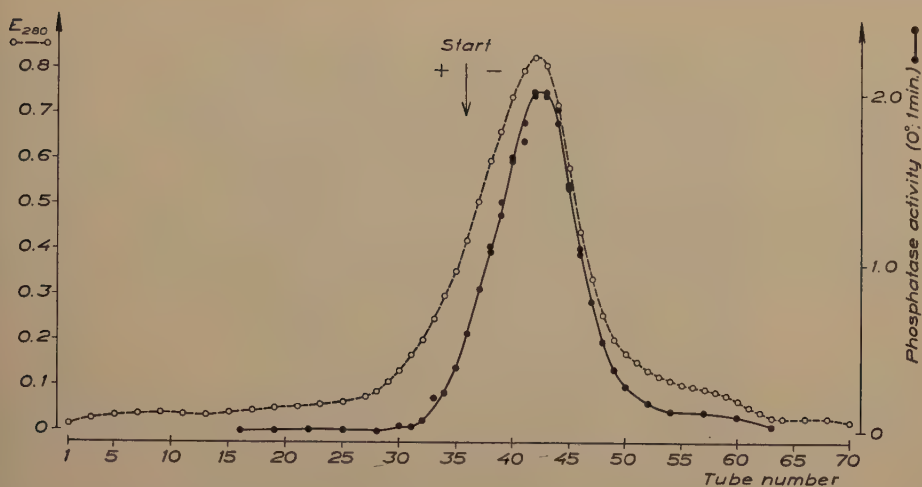


Fig. 6. Electropherogram of prostatic acid phosphatase in citrate-phosphate buffer of pH 4.8. Open circles: Extinction at 280 mμ. Solid circles: Phosphatase activity.

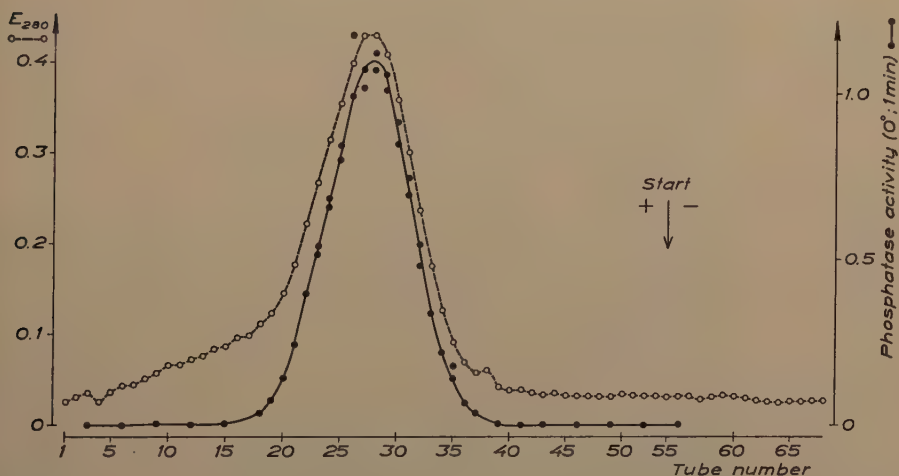


Fig. 7. Electropherogram of prostatic acid phosphatase in citrate-phosphate buffer of pH 7.5. Open circles: Extinction at 280 mμ. Solid circles: Phosphatase activity.

applied was 2 ml with $E_{280} = 24.6$ and the sample had previously been dialysed against the buffer. The run was performed during 16 h with about 45 mA and 1800 V, and for 2 h with 70 mA and 2300 V. The recovery of the material on an activity basis was approximately 70 %. The electropherogram is shown in Fig. 6. In the experiment at pH 7.5, the amount applied was only about half of that used at pH 4.8 and the run was performed during 20 h with about 40 mA and 1600 V. The electropherogram is given in Fig. 7. In both of these electrophoresis experiments, the pump used for the circulation of the cooling ice-water stopped giving a certain temperature rise.

Chromatography

When Fraction II was rechromatographed on Dowex 50 during similar conditions as used in the first run, only one peak was obtained in the chromatogram and the specific activity remained the same (cf. Table 1). In order to study the homogeneity and to remove anion contaminations, Fraction II was chromatographed on DEAE cellulose (21) using gradient elution with a cation buffer. The chromatograms with this adsorbent were not strictly reproducible, possibly depending on an initial denaturation of the phosphatase. Concentrated material from the active zone, however, showed the same specific activity (see Table 1) and the same sedimentation behavior (see Fig. 5) as Fraction II.

Analysis of nitrogen and phosphorus

The nitrogen content has been determined for Fraction II as well as for material which afterwards has been subject to zone electrophoresis and chromatography. These figures are given in Table 1, which also contains data for the extinctions at 280 m μ and the specific activities. The nitrogen content has varied considerably between different preparations and the table gives the highest and lowest figures obtained.

All preparations tested have contained traces of phosphorus. A qualitative investigation of this phosphorus has been done by a method for paper chromatography of phosphoric esters (22), and Fig. 8 shows a photograph of such a chromatogram. In addition to some reference substances (cf. (22)), the chromatogram contains the samples listed in Table 1.

Discussion

The activity determinations

Methods for the determination of prostatic acid phosphatase have usually involved 100- to 20,000-fold dilutions prior to the incubation with the substrate (9, 13, 23). In view of the very apparent surface denaturation during dilution, the present determinations were done with time and temperature decreased as far as convenient. The conditions used do not, however, give activity values which are strictly proportional to the amount of enzyme added (see Fig. 2). This is probably a complex phenomenon, influenced by a too low substrate concentration, errors in the pipetting, and inhibition from the phosphate of the buffers. In a recent study of a highly purified alkaline phosphatase, Portmann (24) gives a discussion of the general difficulties involved in the determination of a very high phosphatase activity. As the situation with prostatic acid phosphatase may be rather similar, and as the possible errors mentioned above must be considered, care ought to be exercised in the interpretation of the activity values. It is believed that the specific activities in Table 1 are suitable for a comparison but no further use will be made of these data.

The denaturation of the enzyme

London and Hudson (9) found that the non-ionic detergent, Tween 80, is an effective protector against the surface denaturation of the phosphatase. For this reason, the present purification was first carried out with a concentration of 0.01 % of Tween 80 during the whole preparation procedure. However, ultracentrifugation showed two peaks for a purified and concentrated enzyme sample with Tween. Separate

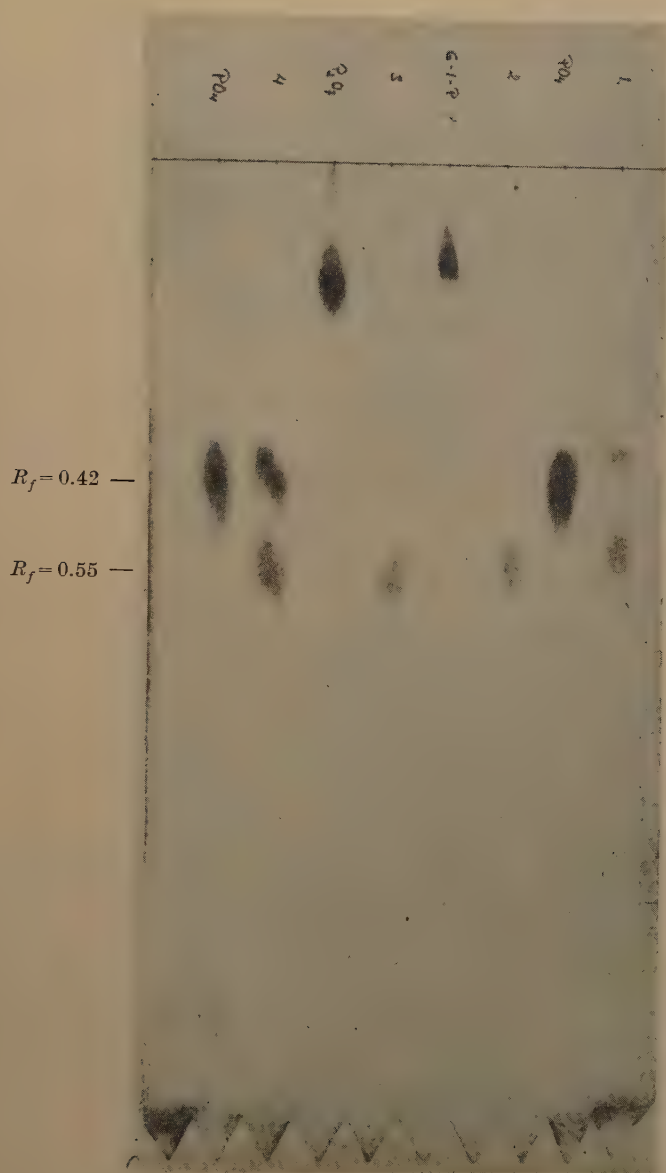


Fig. 8. Paper chromatogram of the phosphorus compounds in four different preparations of prostatic acid phosphatase, denoted 1-4 as indicated in Table 1. The developer was the upper phase of butanol-ethanol-trichloroacetic acid in water (2%) in the ratio 35:10:55. All four enzyme samples contained an unidentified compound with $R_f = 0.55$, and samples 1, 3 and 4 contained in addition varying amounts of orthophosphate which has $R_f = 0.42$. The first and the sixth spot from left shows a standard with about $3 \mu\text{g}$ orthophosphate.

experiments with the Tween only showed the detergent to be dialysable and to have a sedimentation constant of 1.8 S for a 1 % solution in water.

London *et al.* (10) observed that freezing in the presence of large amounts of Tween would inactivate the enzyme. With the procedure used here 0.01 % Tween 80 has protected the enzyme during freeze-drying of Fraction I as well as of Fraction II. Omission of the Tween resulted only in a few per cents inactivation during lyophilization of Fraction I, but in an almost complete inactivation for Fraction II.

Removal of the Tween is achieved in Step 2 during the chromatography on Dowex 50 (cf. also (10)). The stability of Fraction II seems to be rather good for concentrated solutions for which the activity has remained constant over several weeks when kept at 4°. Dilute solutions, however, lose activity and it is thus probable that the 20- to 100-fold dilution, involved in the activity determinations, also causes some inactivation.

Several experiments indicate that inactivated enzyme can show the same chromatographic behavior on Dowex 50 as the material with a specific activity of about 2.5. It is, therefore, of importance to have as fresh a starting material as possible. It appears that large amounts of inactivated enzyme was present in Fraction II obtained from post-mortem material.

The purity of the phosphatase

The ultracentrifugation (Fig. 4) shows that the main part of Fraction II is sedimenting as a homogeneous material giving rise to a narrow and symmetrical peak. Traces of another component can be seen after the main peak, but can hardly represent more than a few per cent of the total material.

In both of the electrophoresis experiments (Figs. 6 and 7) the activity is well associated with the single peak obtained. However, the left sides of the peaks show the presence of some inactive material, which was not detectable in other experiments. It is likely that this represents inactivated phosphatase, produced by the accidentally high temperatures obtained in these runs. It is thus probable that Fraction II represents a prostatic acid phosphatase in about 90–95 % purity. This statement is supported by the fact that the specific activity remains almost the same after the additional fractionations listed in Table 1.

In the electrophoresis experiment at pH 4.8 (Fig. 6), the main part of the zone has moved slightly toward the cathode. The isoelectric point for the phosphatase in citrate-phosphate buffers, is thus a little higher than 4.8. Kutsher and Pany (1) found the isoelectric point in citrate buffers to be 4.4 and Derow and Davison (25) have reported a value of 4.5 in acetate buffers.

The chemical composition of the enzyme

Table 1 shows that the ratio between E_{280} and the nitrogen content is rather high. In case of the values for E_{280} , contamination of soluble resin material must be considered. Such products (polystyrene or sulfonated polystyrenes) would, however, probably be removed during chromatography on DEAE cellulose or during zone electrophoresis at pH 7.2. As the specific activity is not increased by these procedures (see Table 1) and as the recovery of the activity has been 70–90 %, it appears that contamination from resin material cannot make a considerable contribution to the extinction.

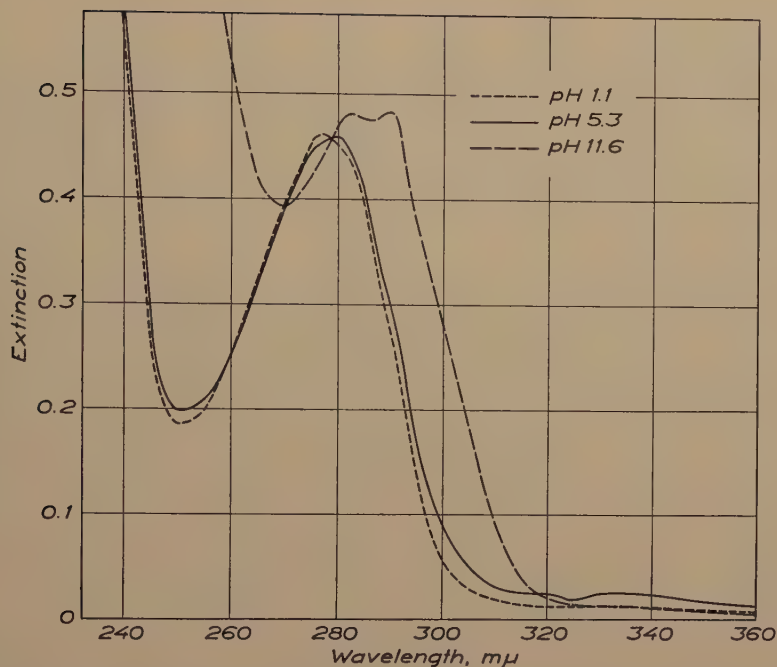


Fig. 9. Ultraviolet absorption spectra for prostatic acid phosphatase at pH 1.1, 5.3 and 11.6. The enzyme used was taken from the tubes with maximum activity in the electrophoresis experiment shown in Fig. 7.

The main reason for too low nitrogen values would be an incomplete Kjeldahl-burning, but as the standard used has been $(C_5H_5N \cdot HCl)_2ZnCl_2$ (16), this explanation is improbable. Earlier findings indicate that ammonium sulfate is adsorbed to the phosphatase (13) and in the present work the use of tris(hydroxymethyl)aminomethane-HCl buffers (THAM-HCl) gave similar results. It is thus possible that the nitrogen concentrations used in Table 1 are too high, rather than too low. Adsorption of low molecular weight compounds with nitrogen, would also account for the differences found between different preparations.

Some additional support for the hypothesis, that the prostatic acid phosphatase adsorbs substances with amino groups, is found in the work of Jeffree (23), which shows that a variety of amino compounds protects the enzyme against surface denaturation.

In Table 1 are included some data from London *et al.* (10) which indicate that their purification also ends with a rather aromatic enzyme. Here, adsorption of ammonium sulfate could explain the differences found between their two methods (9, 10).

It is striking that the extinction coefficient at 280 $m\mu$ (based on dry-weight, about 2.4 for 1 mg/ml in a 1 cm cell) is higher than the values for cytochrome *c* and rhodanese, which have the highest extinctions of the enzymes listed in Table 3.1 of Neilands and Stumpf (26). Fig. 9 shows the spectra of purified phosphatase at three different pH values. The extinctions at 250 and 300 $m\mu$ recorded here at pH 5.3 are significantly lower than the values found by London *et al.* (10) at pH 6. Some contamination of

the form obtained at pH 11.6 in the preparation of London *et al.* (10) would account for these differences.

Fig. 8 shows that all fractions analysed contain a phosphoric ester with an $R_f = 0.55$. In contrast to many phosphoric esters studied (22) this compound moves faster than orthophosphate, which indicates a relatively high solubility in non-polar solvents. However, it remains to show that the substance is related to the enzyme and until this is done further speculations serve no purpose.

Concluding remarks

It is difficult to compare the enzyme preparation obtained here with the one isolated by Lundquist *et al.* (11). However, zone electrophoresis of Fraction I (see Fig. 3 in (15)) shows a pattern quite different from the electropherogram of the seminal plasma (11) and does not give a specific activity higher than 0.7. This indicates that seminal plasma probably is a more well defined material than Fraction I and thus easier to fractionate with electrophoresis.

It is to be expected that the enzyme obtained here, should be similar to the one isolated by London *et al.* (10). The first step in this procedure, the extraction, is a modification of the one recommended by London and Hudson (9), while the chromatography adopted by London *et al.* (10) is a slight variation of the method recommended earlier (12). It appears likely to the author, that the preparation described previously (13) and the one of London *et al.* (10) represent rather pure enzyme, but the evidence, at that time given for the purity may not have been quite satisfactory.

It is too early to draw far reaching conclusions about the chemical composition of the enzyme, but if sufficient amounts of material can be obtained, a detailed investigation of this problem is planned.

SUMMARY

1. A simple purification of prostatic acid phosphatase is described. The first step is an extraction followed by dialysis, the second one is chromatography on Dowex 50.
2. The enzyme obtained is homogeneous in the ultracentrifuge and in electrophoresis and has a pH dependent ultraviolet absorption spectrum with a high extinction coefficient at 280 m μ .

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A deoxyribonuclease from human prostatic tissue

By HANS G. BOMAN

With 5 figures in the text

Introduction

Enzymes which hydrolyse deoxyribonucleic acid (DNA) have been found in very different biological materials and references to older studies can be found in the reviews of Schmidt (1) and McDonald (2). So far, the pancreatic deoxyribonuclease (DNAase I) is the best known of these enzymes, chiefly thanks to the work of Kunitz (3). It appears that the other enzymes known to digest DNA can be grouped into at least three categories, represented by the thymus deoxyribonuclease (DNAase II) (4, 5, 6), micrococcal DNAase (7), and snake-venom phosphodiesterase (8, 9). The main differences, so far established, seem to concern the requirement of, or the inhibition by a divalent metal, the pH range in which the enzyme is active, and the time influence on the viscosity of DNA (9).

Prostatic tissue extracts have been reported to be devoid of diesterase activity (10) but later investigations with crude samples of prostatic acid phosphatase (11) have indicated the presence of diesterase activity. In connection with the isolation of prostatic acid phosphatase (12) it was thus regarded to be of interest to assay different samples for DNAase activity. It was found that different extracts of prostatic tissue contained considerable DNAase activity and that a Tween 80-water extract (12) was a suitable starting material for the preliminary purification and characterisation described in the present communication.

Materials and methods

The starting material used is described in the previous paper (12). The procedure for the purification of prostatic DNAase starts from "Fraction I", the material obtained after the first step in the purification procedure for the acid phosphatase (12).

The *protein content* was estimated as the extinction at 280 m μ (E_{280}) using a Beckman DU spectrophotometer. Water was always used as a blank when reading fractions from chromatographic or electrophoretic experiments.

The *phosphatase determinations* in Fig. 2 were carried out as described in the previous paper (12). The final values for the activities are given as millimoles of *p*-nitrophenol phosphate split per liter of incubation mixture, during 1 min., at 0° with a volume ratio of 1/21 between enzyme and substrate solutions.

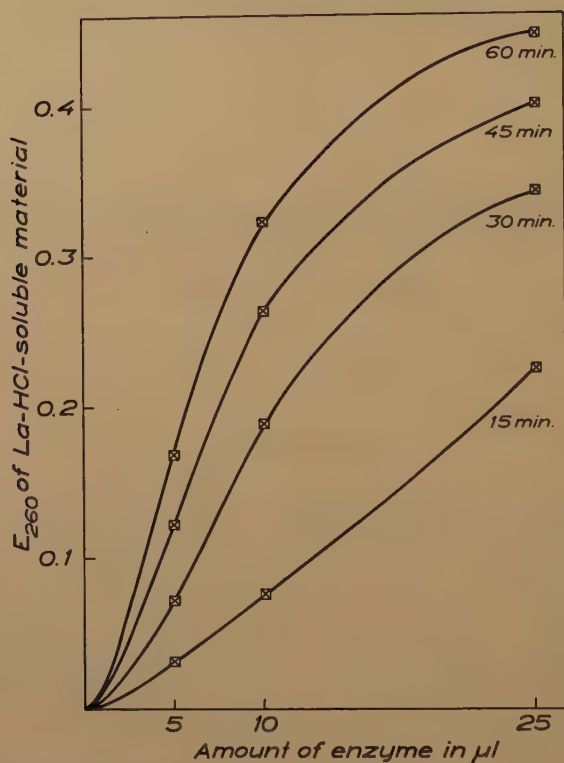


Fig. 1. The liberation of La-HCl-soluble material for different amounts of enzyme (with $E_{260} = 1.2$) and different times of incubation at 37° . The substrate solution (with $E_{260} = 4.6$) contained 0.001 M EDTA and pH was 5.3.

The *DNAase* activity was estimated as the extinction at 260 mμ (E_{260}) of the lanthanum-acid (La-HCl) soluble material obtained after incubation with macromolecular DNA (cf. (8)). The DNA (13) used for the activities given in Figs. 1-3 was dissolved directly in 0.05 M triethylammonium acetate (TEA-HAc) buffer of pH 5.5 (14). The final solution was made 0.001 M with respect to ethylene-diamine-tetraacetic acid (EDTA); pH was 5.3, and E_{260} was 4.62. Of this substrate solution, 200 μl, was incubated with 25 μl of the appropriate fractions during 2 h at 37° . The reaction was stopped with 1 ml of 0.01 M $\text{La}(\text{NO}_3)_3$ in 0.1 N HCl, the precipitated DNA was spun down in a centrifuge and the E_{260} of the supernatant was estimated in a 1 cm semi micro cell of a Beckman DU spectrophotometer. In each set of activity determinations, 4-6 blanks without enzyme were included. The mean value for the E_{260} of these samples has been subtracted from the figures given as the activities. For this substrate solution, the blank has been of the order of 0.1 which shows that about 87 % of the DNA was precipitated. The highest activity estimated with this substrate solution (in Fig. 1) corresponds to about 60 % digestion of the DNA.

For the other activity determinations, a higher substrate concentration was used. It was then necessary to dissolve the DNA (1 mg/ml) in 0.02 N NaOH and afterwards dialyse the solution against the desired TEA-HAc buffer. The blanks

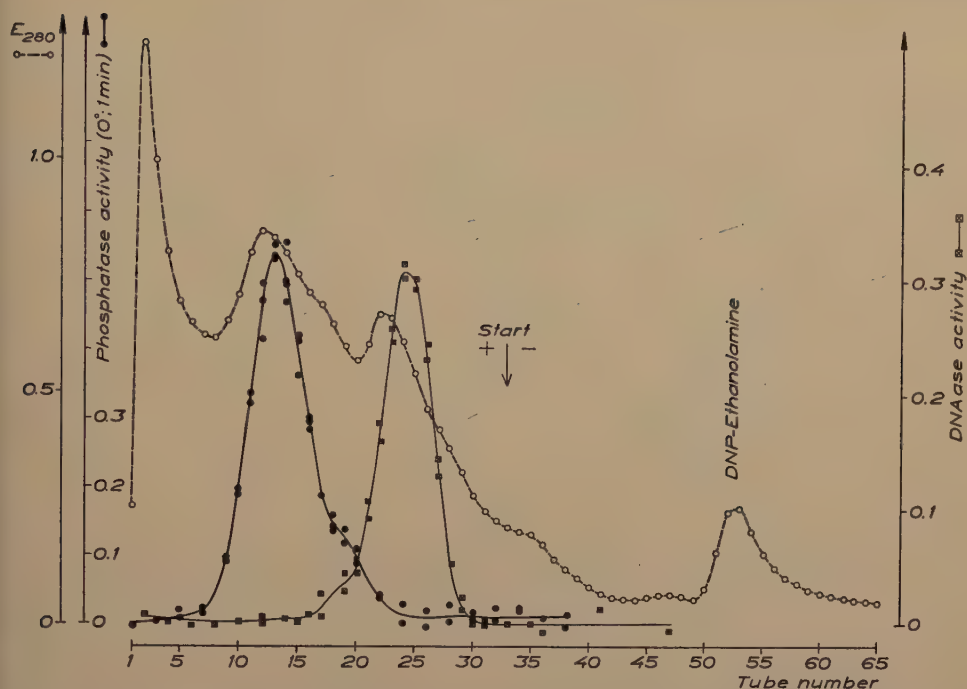


Fig. 2. Electropherogram of about 200 mg of dry prostatic extract in THAM-HCl buffer of pH 8.1. Open circles: E_{280} . Solid circles: acid phosphatase activity. Crossed squares: DNAase activity. Fraction volume around 5.4 ml, except for tubes 1 and 2 which were 9 ml each.

were about 0.08 and the highest activity estimated with this substrate solution (in Fig. 4) corresponds to about 20 % digestion of the DNA.

The *zone electrophoresis* was performed according to the method developed by Flodin and Porath (15, 16), with a column (107 × 2 cm) of ethanolysed cellulose as supporting medium. The buffer used was 0.05 M tris(hydroxymethyl)aminomethane-hydrochloric acid (THAM-HCl) of pH 8.1 with 0.02 M NaCl. Cooling was achieved with circulating ice-water.

The *chromatography* was performed with the DEAE cellulose of Peterson and Sober (17) using a gradient (18) with THAM-HCl buffer of pH 7.7.

Results

Purification procedure

Of the dry prostatic extract denoted Fraction I (12), 250 mg was dissolved in 2.3 ml of electrophoresis buffer of pH 8.1 (see Materials and Methods) and dialysed against the same buffer for a few hours. The undissolved material was removed by centrifugation. Of the supernatant, 2 ml was fractionated by column electrophoresis (15, 16). The experiment was performed during 40 h with about 45 mA and 900 V and the resulting electropherogram is shown in Fig. 2. In this and the following figures open circles represent extinction at 280 m μ , solid circles stand for the acid phosphatase activity and crossed squares denote the DNAase activity recorded as the extinction

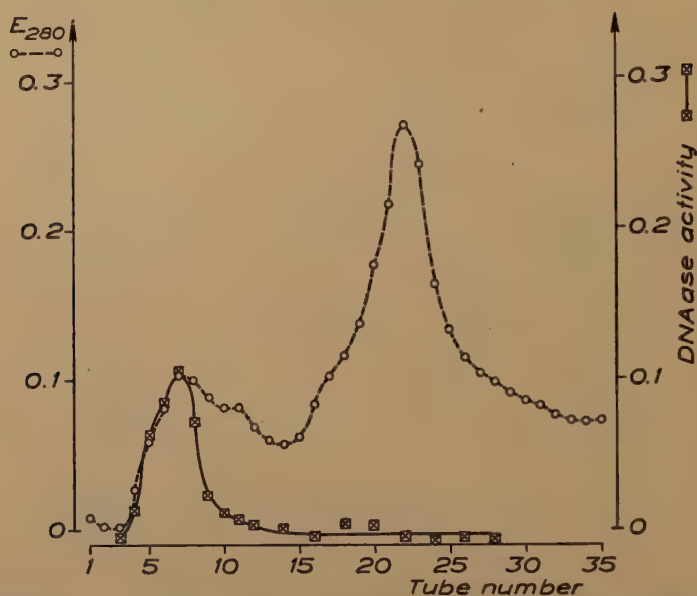


Fig. 3. Chromatogram of DNAase obtained from electrophoresis experiments like the one shown in Fig. 2. Column (18×1 cm) with DEAE cellulose; starting buffer was 0.04 *M* THAM-HCl of pH 7.7, and gradient elution with 0.2 *M* THAM-HCl buffer of pH 7.7. Open circles: E_{280} ; crossed squares: DNAase activity. Fraction volumes around 5 ml.

at 260 $m\mu$ of the La-HCl-soluble material obtained after incubation with macromolecular DNA. The first zone obtained in this electrophoresis (tubes 1–2 in Fig. 2) has a brownish color and is in this figure too narrow which depends on the large volume of these two fractions. In addition to the zones shown in Fig. 2, Fraction I contains some components, which move faster than the brownish material and which in the present experiment were allowed to pass out in the anode vessel. The zone with the DNAase activity tubes 21–19 in Fig. 2) was pooled together with the enzyme from a similar experiment and used for the next step, a chromatographic fractionation.

Of the pooled DNAase solution, 19 ml was dialysed against 0.04 *M* THAM-HCl buffer of pH 7.7 (the starting buffer). The enzyme solution was applied to a column (18×1 cm) with DEAE cellulose (17) and afterwards a few milliliters of starting buffer was passed into the column. The chromatogram was then developed with a concentration gradient toward 0.2 *M* THAM-HCl buffer of pH 7.7. As Fig. 3 shows, the DNAase activity appears in the first zone (tubes 4–10). However, the recovery of the activity was poor, only about 35 %. The enzyme in tubes 4–7 was concentrated by negative-pressure dialysis (19) and used in the following study of the enzymic properties.

Enzymic properties of prostatic DNAase

Preliminary experiments for the determination of the pH optimum of the enzyme indicated a high activity around pH 5, while at pH 6.3 and 8.2 no digestion of DNA was observed. A more detailed determination was carried out in the following way:

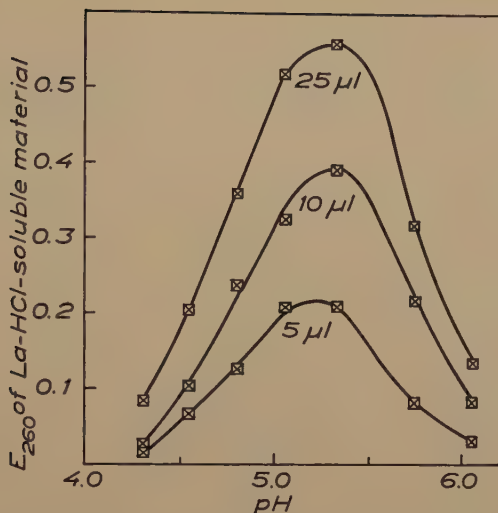


Fig. 4. The pH optimum for prostatic DNAase. Different amounts of enzyme (with $E_{280} = 1.2$) were incubated for 2 h at 37° with $200\ \mu\text{l}$ of 0.1 % DNA in 0.1 M TEA-HAc buffers.

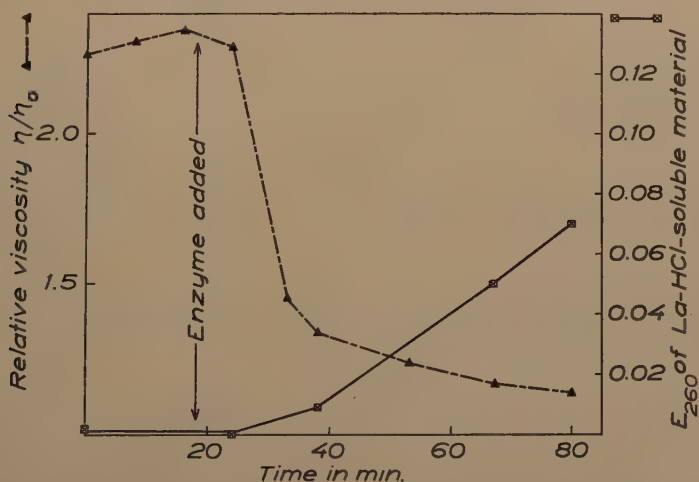


Fig. 5. The relation between the relative viscosity (solid triangles) and the liberation of La-HCl-soluble material (crossed squares) for 5 ml of 0.1 % DNA in 0.1 M TEA-HAc buffer of pH 5.3 to which $100\ \mu\text{l}$ of prostatic DNAase (with $E_{280} = 1.2$) was added after 18 min. The experiment was carried out at 37° in an Ostwald viscometer with a flow time of 141 seconds for water.

A 0.1 % solution of DNA, dissolved in 0.02 N NaOH, was divided into three parts. Each part was dialysed against a 0.1 M TEA-HAc buffer, the pH being different: 4.2, 5.7 and 9.9, respectively. Mixtures of these three dialysed solutions of DNA were used for the activity determinations given in Fig. 4. Of the substrate solutions, $200\ \mu\text{l}$ were incubated with 5, 10 and $25\ \mu\text{l}$ enzyme ($E_{280} = 1.2$), during 2 h at 37° . Blanks were taken from the solutions of pH 4.30, 4.80, 5.33 and 6.05 and varied between 0.086 and 0.079.

Table 1. Effect of ions on prostatic DNAase activity.

Addition	Final conc. in <i>M</i>	Activity in %
—	—	100
Na ₂ SO ₄	0.008	9
	0.02	0
(NH ₄) ₂ SO ₄	0.008	19
	0.02	0
MgSO ₄	0.004	23
	0.008	0
MgCl ₂	0.008	30
	0.02	6
NaCl	0.016	83
	0.04	58
EDTA	0.002	178
	0.005	175
{ EDTA }	{ 0.01 }	28
{ MgSO ₄ }	{ 0.008 }	
Citrate, pH 5.0	0.02	60

In order to obtain information about the type of action the enzyme shows toward macromolecular DNA, the viscosity of an incubation mixture was followed simultaneously with the liberation of La-HCl-soluble material. Of a 0.1 % DNA in 0.1 *M* TEA-HAc buffer of pH 5.1, 5 ml was pipetted down in an Ostwald viscometer with a flow time of 141 sec. for water at 37°. After 18 min. of incubation, 100 μ l of DNAase (with $E_{280} = 1.2$) was added to the DNA solution in the viscosimeter. At different times, samples of 200 μ l were removed from the incubation mixture for an immediate precipitation with 1 ml of 0.01 *M* La(NO₃)₃ in 0.1 *N* HCl. The experiment was continued for 80 min. and the results are shown in Fig. 5, where the E_{260} of the La-HCl-soluble material is denoted with crossed squares and the relative viscosity with solid triangles.

Some salts and two substances known to form complexes with divalent cations, have been tested for their influence on the DNAase activity, and the results are shown in Table 1.

Discussion

The data given in Fig. 1 show that, for a sufficient incubation time, the liberation of La-HCl-soluble material is approximately proportional to the amount of DNAase. The substrate concentration used, however, will not permit the estimation of activities higher than about 0.3, which corresponds to a hydrolysis of 40 % of the substrate. As Figs. 2 and 3 do not include any activities which exceed this magnitude, it appears that these graphs give a relatively accurate picture of the distribution of the DNAase activity in the fractionations used here.

The results given in Fig. 5 indicate that viscosity is a much more sensitive method for the estimation of prostatic DNAase than is the liberation of La-HCl-soluble material. However the viscometric method for the estimation of DNAase (20), is not convenient for large series of determinations, and moreover, in the case of chromatographic fractions, differences in salt concentration may cause serious errors. In the case of high DNAase activities, as with relatively pure enzymes, the lower

sensitivity of the "La-HCl-soluble" method may be an advantage, but the *absence* of prostatic DNAase, must be checked with the viscometric method.

The purification procedure used should be regarded as only a preliminary one. A main reason to start with electrophoresis was the successful purification of seminal plasma enzymes achieved by Lundquist *et al.* (21). However, a comparison of the electropherograms in Fig. 2 of this communication and Fig. 3 of Lundquist *et al.* (21) shows that the starting materials are quite different, also when considering that the starch used as supporting medium can produce some differences in the separation pattern when compared to the ethanolyzed cellulose (15) used here. In view of the general experiences of the author, it is preferable to start a purification with chromatography, which, however, in this particular case was unsuitable, depending on the low recovery of the enzyme after chromatography on DEAE cellulose.

During a study of the phosphodiesterase from rattle-snake venom (22) it was found that this enzyme liberated La-HCl-soluble material, despite the fact that no DNAase activity had been found with the viscometric method (23). As suggested by Laskowski *et al.* (9), this indicates the cleavage of diester bonds at the end of a polynucleotide chain, and the phosphodiesterase of the rattle-snake venom may thus be regarded as an exonucleoidase. The experiment with the simultaneous recording of viscosity and La-HCl-soluble material of an incubation mixture (Fig. 5) shows that with prostatic DNAase the viscosity of macromolecular DNA is decreased prior to the appearance of La-HCl-soluble material. This indicates that the primary effect of prostatic DNAase is a depolymerization of the DNA.

The role of divalent cations for enzymic hydrolysis of DNA is an interesting problem. Both the pancreatic DNAase (3) and the DNAase II from thymus (5, 24) require Mg for full activity, but inhibition occurs for higher concentrations of Mg, a fact utilized in the preparation of nucleoprotein (25). It is possible that the *in vivo* function of the "intracellular" enzymes can be regulated by the metal-ion concentration, an hypothesis supported by the influence of Ca and Mg on the natural state of DNA (26). Table 1 shows that the addition of EDTA causes an increase in the DNAase activity, possibly due to the removing of traces of some inhibitory metals. As a non-metal buffer was used, and as the DNA was pre-incubated with EDTA, it appears unlikely that prostatic DNAase requires a dissociable metal ion. Fig. 4 shows that the pH optimum of the enzyme is around 5.2, which is similar to the value for DNAase II. Judging, however, from the behaviour toward EDTA and Mg (see Table 1), it appears that the prostatic DNAase may differ from DNAase II and other "intracellular" DNAases (5, 24, 27). The results given in Table 1 indicate also that prostatic DNAase is inhibited by sulfate ions (cf. (24)), which may be an explanation why it has not been detected in the purification of prostatic acid phosphatase with ammonium sulfate precipitation (28, 29).

It is hoped to continue the purification of prostatic DNAase and to make a more detailed study of the enzymic properties when a pure and homogeneous enzyme is available.

SUMMARY

1. Zone electrophoresis and chromatography have been used for a partial purification of a deoxyribonuclease from an extract of human prostatic tissue.

2. The enzyme has pH optimum around 5.2 and causes a depolymerisation of macromolecular deoxyribonucleic acid. The activity of the enzyme is not inhibited by 0.005 *M* ethylene-diamine-tetraacetic acid but complete inhibition was obtained with 0.02 *M* sulfate.

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The infrared spectra of some vitreous and crystalline borates

By J. KROGH-MOE

With 4 figures in the text

Introduction

Several authors have studied glass structures by means of infrared absorption spectra. Such studies have often been based on an assignment of peaks in the spectra to definite structure elements like BO_4 , BO_3 and SiO_4 groups. This procedure is rather simplified, and warnings against it have appeared (1). Nevertheless new attempts along these lines have been published in the last years. A brief review of some relevant aspects of the basic theory shall therefore be given in the following.

An isolated assembly of N atoms can have as many as $3N-6$ different fundamental modes of vibration. Each of these modes may give rise to an absorption line in the infrared spectrum. In many cases the number of fundamental absorption lines will be smaller than $3N-6$ because of symmetry relationships within the assembly. The spectrum of a molecule having more than 5 to 10 atoms, however, will usually be so complicated that a complete assignment of experimental absorption lines to modes of vibration is exceedingly difficult.

A fundamental vibration in an assembly of atoms involves the simultaneous vibration with the same frequency of all the oscillating atoms. The fundamental vibration will therefore not be characteristic of any structural sub-unit except in special cases when this unit is weakly coupled with its surroundings. Weak coupling occurs when widely different forces or masses are present. For instance molecules condensed in a crystal lattice by weak van der Waals forces will have nearly the same infrared absorption spectrum as the free molecules in gas phase.

Vibrations of structural units like SiO_4 and BO_4 tetrahedra or BO_3 triangles in a glass will generally be strongly coupled with the surroundings. These units are usually connected to a three-dimensional network and the infrared spectrum is therefore characteristic of a certain region in this network. In some cases the glass has a structure similar to a crystalline phase. Unit cell dimensions of this crystalline phase will then indicate the order of magnitude of the region in the glass which determines the main features of the near infrared spectrum.

A continuous distribution of fundamental frequencies are usually observed in a glass. Peaks in this distribution will be broader in a glass than in a comparable crystalline phase, indicating that the surroundings of any group in the glass do not repeat in a regular fashion. A comparison of such glass and crystal structures will be made in the present paper.

The complexity of the infrared spectrum of borate glasses prevents that much information about the glass structure can be obtained from this source. It will be shown, however, that some conclusions may still be drawn from the infrared data.

Experimental

Samples for the infrared investigation were prepared by melting down the appropriate chemicals (like boric acid, borax and sodium carbonate) in a platinum crucible and raising the temperature to 1000°C for dehydration. Merk p.a. reagents were used in all cases except in the systems containing lithium or cesium. Here lithium carbonate and cesium carbonate labeled as purum was used for preparing the samples. The sample of cobalt pyroborate was of unknown origin and purity, but the identity was ascertained with an X-ray powder pattern.

The spectra were studied by means of the potassium bromide briquette technique. From 0.5 to 4 milligram of the sample was grinded down with 2 gram of potassium bromide. In the case of boron oxide glass, this grinding was done in a dry chamber, since the glass is extremely hygroscopic. The powder mixture was pressed to a clear, circular disc of approximately 2.5 cm radius by applying a nominal pressure of 4000 kg/cm² for 15 minutes to the evacuated pressure chamber.

The spectra were recorded with a Perkin Elmer 21 (double beam) spectrometer in the interval from 2 to 15 microns, using a sodium chloride prism. The results are shown in Figs. 1, 2, 3, and 4.

Discussion

Fig. 1 shows the infrared spectra of an orthoborate, a pyroborate and a metaborate, for which the structures are known.

For some orthoborates, including boric acid, a complete assignment of infrared absorption lines to fundamental modes of vibration have been made (2, 3). The spectrum of boric acid shown on top in Fig. 1 agrees very well with previous results (2, 4). Lines in addition to those expected for free BO_3^{3-} ions are present. These lines, caused by the presence of the hydrogen, are found at approximately 3.2 and 8.4 micron.

The spectrum in the middle of Fig. 1 is that of crystalline cobalt pyroborate. Berger has shown the existence of discrete $\text{B}_2\text{O}_5^{4-}$ ions in this compound (5). The ion consists of two BO_3 triangles sharing a corner. The two planar BO_3 groups are twisted and bent with respect to each other, so that the ion has no center of symmetry. This increases the number of infrared active fundamental vibrations. Though the ion is fairly small, the spectrum has many and poorly resolved lines.

The lower curve in Fig. 1 shows the spectrum of crystalline sodium metaborate. In this compound the anion is known to consist of ringshaped $\text{B}_3\text{O}_6^{3-}$ groups (boroxol rings) built from planar BO_3 triangles sharing corners (6). Three investigations of the infrared spectrum of sodium metaborate reported previously by different authors do not mutually agree very well (4, 7, 8). The present result can largely be reconciled with that of Duval and LeComte, however (8). Also, there is a considerable resemblance to the spectrum of vitreous lithium metaborate reported in this paper. (See Fig. 3.)

From what has been said it appears that all the compounds represented in Fig. 1 have structures based on the BO_3 group. On connecting these groups, a number of bands appear in the region from 7 to 12 micron. Thus from the comparison in Fig. 1 follows that the extent to which BO_3 triangles are combined to larger groups have a pronounced effect on the spectrum.

The spectra of boron oxide, sodium tetraborate ($\text{Na}_2\text{O} \cdot 4\text{B}_2\text{O}_3$) and sodium diborate ($\text{Na}_2\text{O} \cdot 2\text{B}_2\text{O}_3$) are shown in Fig. 2. Solid lines refer to glass and dotted lines to crystals of the same compositions. The crystalline boron oxide was identified as the hexagonal modification by its X-ray pattern. Berger has determined the structure of this

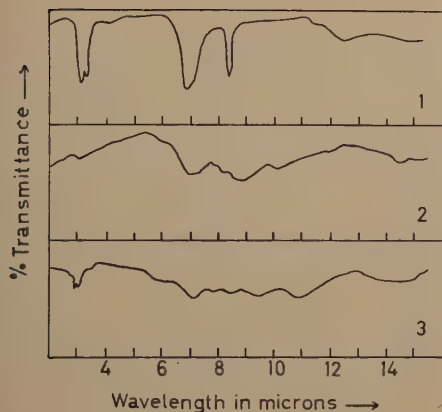


Fig. 1. The infrared spectra of some crystalline borates. 1, boric acid; 2, cobalt pyroborate; 3, sodium metaborate.

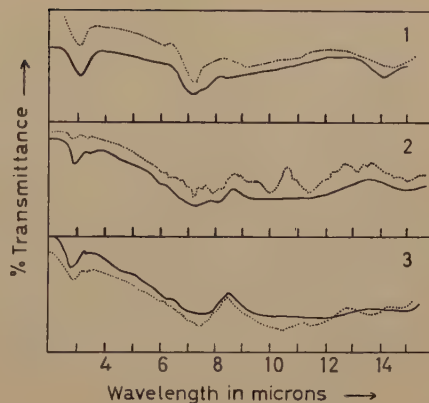


Fig. 2. The infrared spectra of three compounds in the system boron oxide-sodium oxide. Dotted lines are for the crystalline phases, solid lines refer to glass. 1, boron oxide; 2, sodium tetraborate ($\text{Na}_2\text{O} \cdot 4\text{B}_2\text{O}_3$); 3, sodium diborate ($\text{Na}_2\text{O} \cdot 2\text{B}_2\text{O}_3$).

modification and found it to be built up of distorted BO_4 tetrahedra (9). The unit cell dimensions of the crystalline sodium tetraborate have been given by Krogh-Moe (10).

An inspection of the spectra in Fig. 2 reveals a strong correlation between the corresponding curves of glass and crystal. Boron oxide glass differs mainly from the crystalline boron oxide by a shift of the 14.5 micron peak to 14 micron. Nevertheless the glass and the crystalline phase must have different structures, since they have very different densities. The difference in densities is probably related to the degree and amount of four coordination of boron. Apparently this coordination change will not strongly affect the spectrum.

A comparison of the infrared spectrum of boron oxide glass with the raman spectrum is also of some interest. The strongest line in the raman spectrum correspond to a frequency shift of 809 cm^{-1} (11). No counterpart (at 12.4 micron) is observed in the infrared spectrum given in Fig. 2. Hence the line is probably due to a symmetrical mode of vibration since it is inactive in infrared. This agrees with a suggestion by Goubeau and Keller that boron oxide glass contain boroxol units (12). These authors found a very strong raman line around 800 cm^{-1} in compounds based on boroxol rings. Boroxol rings are hexagons with boron and oxygen occupying alternate corners. The raman line in question was assigned to a symmetrical vibration of the three oxygens belonging to the ring. This vibration will only be weakly affected by coupling through the three linkages connecting the boron of the hexagon to the surroundings. The weak coupling is expected because the atoms in the connecting linkages are not involved in the vibration. The assignment of this frequency in boron oxide therefore seems reasonable.

Crystalline sodium tetraborate exhibits a spectrum with considerably more details than the glass (see the middle curve in Fig. 2). A spectrum very similar to the glass spectrum would result, however, if the bands were broadened. The band at 2.9 micron in the glass is probably due to residual water, which escapes during the crystallization.

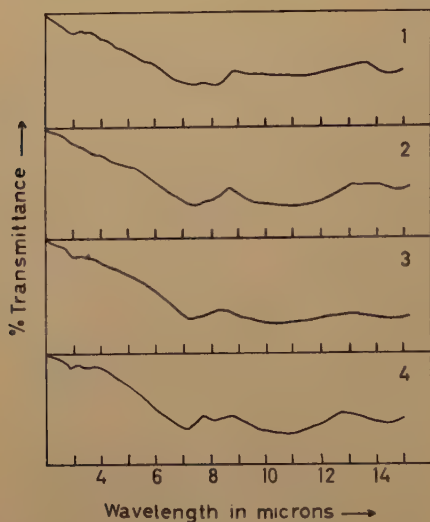


Fig. 3. The infrared spectra of some lithium borate glasses. 1, 18.0 mole % Li_2O ; 2, 27.6 mole % Li_2O ; 3, 40.0 mole % Li_2O ; 4, 50.0 mole % Li_2O .

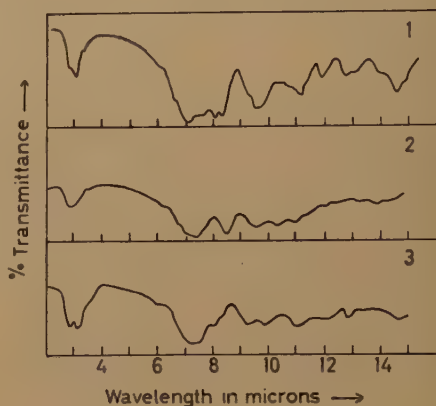


Fig. 4. The infrared spectra of some crystalline cesium borates. 1, cesium pentaborate; 2, cesium tetraborate; 3, cesium triborate.

The infrared absorption spectra of boron oxide glass and some alkali borate glasses have been investigated by Anderson, Bohon and Kimpton (7) and by Moore and McMillan (13). Jellyman and Procter have studied the infrared reflection spectra (14). The absorption curves reported for boron oxide glass differ somewhat from the result obtained in the present investigation. (If care was not taken to grind the boron oxide in a dry box, a curve like that reported by Anderson *et al.* was obtained.) The spectra reported previously for the sodium borates, however, agree reasonably well with the present results. The addition of sodium oxide to boron oxide results in several new infrared absorption bands in the region from 7 to 14 micron. This have been taken by several authors to indicate the formation of BO_4 tetrahedra (7, 13, 14). For instance bands at 7.5, 9.5 and 10.5 micron were attributed to the BO_4 groups, arguing that these are the only new groups which are developed in the alkali borate glass (13). Such arguments overlook the fact demonstrated in Fig. 1 that new bands will also appear if BO_3 triangles are interlinked in a different manner. Furthermore the spectrum of crystalline boron oxide does not exhibit the set of bands at 7.5, 9.5 and 10.5 micron even though BO_4 tetrahedra do exist in this phase.

In Fig. 3 are given the spectra of some lithium borate glasses. A strong resemblance to the corresponding curves in Fig. 2 is evident. This indicates that the boron-oxygen network in lithium borate glasses is similar to the network in sodium borate glasses of the same molar composition. This conclusion have been made also by previous investigators (13, 14). An absorption band in the neighbourhood of 3 micron in the sodium borates, probably due to a hydroxyl stretching vibration, is rather inconspicuous in the lithium borates. The fact that lithium borate glasses are far less hygroscopic than the sodium borate glasses may account for this.

Fig. 4 shows the spectra of some crystalline cesium borates. From top to bottom in Fig. 4 are given the spectra of $\text{Cs}_2\text{O} \cdot 5\text{B}_2\text{O}_3$, $\text{Cs}_2\text{O} \cdot 4\text{B}_2\text{O}_3$ and $\text{Cs}_2\text{O} \cdot 3\text{B}_2\text{O}_3$. Crystallographic properties of these borates have been studied by Krogh-Moe (15). A large number of bands occur in the spectrum. The details of the curves vary considerably from one compound to the next. The majority of the bands can therefore not be looked upon as characteristic of simple BO_3 triangles or BO_4 tetrahedra. Rather such bands depend upon the spatial distribution of bridging and nonbridging oxygens in the anion network. (The existence of BO_4 groups at all in these alkali borate glasses is a conjecture.) If a broadening of the bands in Fig. 4 took place, again spectra similar to those of the corresponding lithium and sodium borate glasses would emerge. Jellyman and Procter have shown that the infrared reflection spectra of the cesium borate glasses change with composition in much the same manner as the other alkali borates (14).

It is apparent that few conclusions can be drawn from the infrared spectra of the borate glasses at the present stage. The results given here have shown that the broad absorption bands in glass may be due to several superimposed bands, broadened by the disorder in the glass. Caution in making any assignment of the broad absorption bands in glass is therefore required.

SUMMARY

Infrared absorption spectra of a number of crystalline and vitreous borates have been determined in the range from 2 to 15 micron. It is shown that some previous interpretations of the spectra of borate glasses should be reconsidered. No verification of the existence of BO_4 tetrahedra in the glass have been obtained from the infrared data. The spectra obtained indicate a structural relationship between some crystalline and glass phases of the same composition. Furthermore it was found that lithium and sodium borate glasses of the same molar composition had similar spectra, also indicating a close resemblance of the structures.

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Uptake of radiosulfate and radiophosphate in various tissues of normal and vitamin C-deficient guinea pigs

By ULF FRIBERG

With 3 figures in the text

Wolbach and Howe (67) characterized scurvy as a disease in which the formation and maintenance of intercellular substances by the supportative tissues are deficient. This conclusion is corroborated in reference to collagen by the work of Höjer (35), Hunt (40) and Pirani and Levenson (54). Other authors have presented evidence suggesting an interference with the rate of production of the ground substance mucopolysaccharides or suggesting the synthesis of abnormal mucopolysaccharides, in this condition (10, 32, 52, 53). Of the mesenchymal mucopolysaccharides, chondroitinsulfuric acid (CSA) has recently been extensively studied with the aid of S^{35} sulfate by Dziewiatkowski (22), Boström (3) and others. When administered to an animal in this form, the bulk of the radioisotope is rapidly excreted (20, 27). The lesser part, withheld in the body, is assumed to be incorporated mainly into the sulfomucopolysaccharides and other ester sulfates (3). A logical corollary would therefore be to study the effect of scurvy on the uptake of radiosulfate by the animal organism. In 1954, Reddi and Norström (55) reported that the incorporation *in vivo* of radiosulfate into CSA of costal cartilage of scorbutic animals, was reduced to about one-third of that in the normal animals. Simultaneously and independently the present author, in collaboration with Ringertz (31), showed that in vitamin C-deficient guinea pigs the uptake of radiosulfate was reduced to about one-third in most of the tissues examined. On the other hand, scurvy did not cause a lowered uptake of P^{32} phosphate except in bone. These two papers have clearly demonstrated that acute scorbutus is associated with a reduced uptake of radiosulfate in the guinea pig tissues, and this finding has been repeatedly confirmed (34, 42, 55, 66).

The aim of the present investigation was to study the uptake of radiosulfate in various tissues as well as its incorporation into CSA/A in guinea pigs at different stages of vitamin C-deficiency (experiment A). It was also considered of interest to augment and to confirm the preliminary observations of P^{32} retention, mentioned above (experiment B).

Material and methods

General procedures

Growing guinea pigs of both sexes were used. They were kept in the laboratory for two weeks prior to the start of the experimental treatments. Animals that showed any signs of disease or failed to gain weight at a normal rate were discarded. The average weight after the acclimatization period was about 230 g. The guinea pigs were housed in net-bottom cages in a room with a thermostatically controlled temperature of approximately 22°C. The room was lighted between 6 a.m. and 6 p.m. by fluorescent tubes and was equipped with an ultraviolet tube, continuously burning. No other experimental animals were in this room. All animals received the same vitamin C-free basal diet consisting of:

Crushed oats	30 parts	Dried brewer's yeast	5 parts
Wheat bran	28	Salt mixture (38)	2
Hay, chopped	20	Total	100
Whole milk powder	15		

The hay and the milk powder were heated in shallow trays at 110°C for 24 hours in an aerated oven. The diet was made up in portions of 50 kg. The animals received this diet *ad libitum* in two feedings per day. They had free access to drinking water. Normally, they ate around 25–35 g and drank about 40–50 ml per day. As a vitamin supplement, the animals were given, once weekly, 0.4 ml per os of an emulsion that contained, per 100 ml:

Ol. jecoris forte Ph. Suec. XI	2.917 g	Menadiol tetrasodium phosphate	1.459 g
Ol calciferoli conc. Ph. Suec. XI	0.365	Rutin	2.917
α-tocopherol acetate	5.834	Cyanocobalamin	0.0015

together with certain amounts of ground-nut oil and Tween 80 (Atlas Powder Co., Inc., Wilmington, Del.). Allowing for about one-third spillage and unresorbed material, a daily dose of 60 IE vitamin A, 24 IE vitamin D₂, 2.4 mg vitamin E, 0.6 mg vitamin K, 1.2 mg "vitamin P" and 0.6 μg vitamin B₁₂ was assured. A supplement of vitamin C was given during the two weeks preceding the experiments, and to all control animals throughout the investigation, by adding ascorbic acid to the drinking water to a concentration of 0.8 per cent. During the first week after arrival at the laboratory, the animals also received two 50 mg doses of vitamin C per os. The weight of the animals was checked twice a week.

The animals were sacrificed by severance of the jugular veins under ether anesthesia. Blood samples were obtained in conjunction with the exsanguination. Specimens were taken from several tissues: liver, spleen, kidney, intestine (jejuno-ileum), brain, skin (dorsal), muscle (thigh), bone (femur diaphysis), teeth (incisors), cartilage (rib) and intervertebral disc.

Diagnosis of scurvy

The development of scurvy can be conveniently followed by studying the weight curves plotted for the individual animals. Guinea pigs on a vitamin C-deficient diet ceased their growth after about 10 days. For a few days after this, the weight remained constant but then fell precipitously. Furthermore, other clinical signs could be observed, including impeded motion, diarrhea, anorexia, etc. These signs developed

during the third week on the deficient ration. At post mortem examination, periarticular hemorrhages, increased brittleness of bones, and mucinous consistency of connective tissue, were observed. Roentgen examination of the costochondral junctions according to Göthlin (33) was carried out in experiment A. The anterior rib cage and sternum were removed from the animal, then pressed flat against Kodirex X-ray film packs (Kodak, Ltd., London, England). Exposures were made at 65 kV with a Model B dental x-ray apparatus (Ritter Co., Rochester, N.Y.), with a tube to sample distance of 155 cm. Films were developed in D 19 b. The presence on the roentgenograms of transverse hypercalcified epiphyseal lines was chosen as the arbitrary indicator of acute scorbutus. In experiment A assay of the ascorbic acid levels in fresh whole blood was also carried out, according to the method of Roe and Kuether (60), as described by Friberg and Åberg (30). The method involves oxidation of the ascorbic acid in a metaphosphoric acid filtrate with 2,6-dichlorophenolindophenol. Excess dye is removed by thouraea, and the samples are incubated with 2,4-dinitrophenylhydrazine at 37°C for 3 hours. After cooling, sulfuric acid is added, and the developed color read at 520 m μ in a model B spectrophotometer (Beckman Instruments Inc., South Pasadena, Cal.). This method permits analysis of samples containing down to 1 μ g of ascorbic acid per milliliter.

Preparation of CSA

CSA was prepared from the costal cartilage according to the method of Boström and Månsson (5, 6). In the present study, sufficient material was not obtained to permit a complete analysis of the CSA. It has previously been shown, however, that with this method CSA of a high degree of purity is obtained (5, 19, 58). In a subsequent paper (29), analytical data for guinea pig CSA will be presented. Radioactivities were determined on intact CSA.

Tracer technique

Carrier-free solutions of S³⁵ sulfate and P³² phosphate were obtained from the Radiochemical Centre (Amersham, England). The isotopes were administered in single intraperitoneal injections 48 hours prior to death. Radiosulfate was given in a dose of 1 μ c/g body weight and radiophosphate in a dose of 0.1 μ c per gram.

Blood and tissue specimens were collected in small nickel-plated cups (type E-5, Tracerlab Inc., Boston, Mass.) and were dried to constant weight at 50°C. The tissues were then pulverized, and plated on brass discs in a layer 1 mm thick and with a circular surface area of 1 cm². The radioactivities were determined with a Tracerlab TGC-2 G-M tube shielded by 2" of lead, the sample to window distance being 3.5 mm. Samples containing S³⁵ were all determined at infinite thickness, and P³² samples within the narrow range of 60–90 mg/cm². Therefore, no corrections were made for self-absorption. The usual corrections for coincidence, background and decay were, however, carried out (44, 62). The activities were expressed as cpm/cm² for radiosulfate, and as cpm/cm²/mg dry tissue for radiophosphate.

The plating error was determined in the following way. Twenty discs were filled from the same batch of radiosulfate containing liver powder, and each sample was counted during 1 minute. The average rate was 1834 cpm and the standard deviation amounted to 4.3 per cent. With a counting error of 2.3 per cent, the error attributable to plating was then 3.7 per cent. A total plating and counting error of less than 5 per cent could thus be achieved by registering at least 1000 counts for each sample.

Statistical treatment

Means, standard deviations, regression equations, etc. were computed according to conventional methods (28). Significances of differences of the means were determined with Student's *t* test. The probabilities are denoted by asterisks (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$).

Experiments and results**A. Uptake of radiosulfate**

This experiment was conducted on a total surviving number of 74 deficient animals and 52 normal controls. It was carried out in two similar series with an interval of 10 days, referred to as 1 and 2 respectively in the tables. Twenty-seven of the deficient animals died during the experiment, corresponding to a mortality, in the 23 day groups, of 55 per cent in series 1 and 40 per cent in series 2. These animals were discarded, as were three control animals that died.

The mean weight curves of the guinea pigs are plotted in Fig. 1. The initial weight averaged 230 g with a standard deviation of ± 30 g. The deficient animals began to lose weight after approximately 14 days on the scorbutogenic ration. The animals were killed after 10, 17 and 23 days. The blood ascorbic acid concentration of the deficient animals was 1 $\mu\text{g}/\text{ml}$ or less. In the normal animals the level was 3–6 $\mu\text{g}/\text{ml}$. The Göthlin sign was present in all deficient animals examined at 23 days and, in a few instances, already by 17 days. The normal animals did not show this sign (Fig. 2).

Tables 1, 3 and 5 show the results of the radio-assays. The radioactivity varied considerably between the individual animals of a group. The coefficients of variation usually were about 30–40 per cent, but sometimes amounted even to 90 per cent. A cursory examination indicated a positive correlation of the amounts of radioactivity in the different tissues to each other. To validate the degree of correlation a formal regression analysis was performed, by comparing all tissues to liver (Tables 2, 4, 6). The average uptake of S^{35} in the normal tissues varied from 1.8 for cartilage and intervertebral disc down to 0.2 for teeth and brain, with the mean liver radioactivity of each group of animals taken as unity. There was an obvious correlation between the

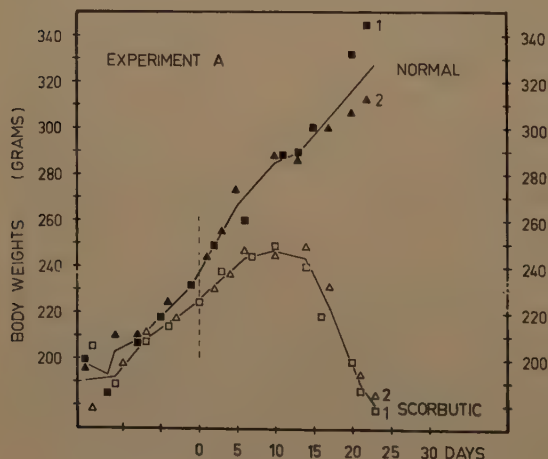


Fig. 1. Mean weight curves, experiment A. Weight at start of experiment 232 g. Coefficient of variation 10–15 per cent; 20 per cent for the 23-day scorbutic group. Animals on the deficient diet began to lose weight after approximately 14 days.

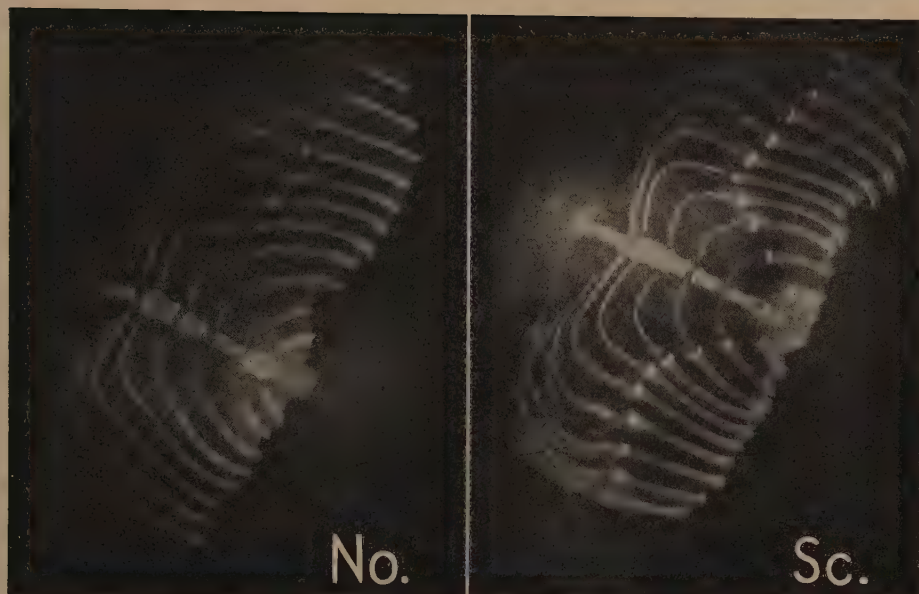


Fig. 2. Roentgenograms of the rib cage of one normal (*No.*) and one scorbutic (*Sc.*) guinea pig from experiment A, both animals killed after 23 days. The scorbutic animal presents the Göthlin sign: hypercalcified, transverse epiphyseal lines at the costochondral junctions.

mean weight loss of the deficient animals and the mean tissue radioactivities at 10, 17 and 23 days. However, attempts to correlate the S^{35} activity of the tissues in the individual deficient animals at 23 days with ascorbic acid concentration in blood or with the weights or weight changes were unsuccessful. The results of these calculations were not included in the tables.

Ten days

Table 1 shows that the uptake of S^{35} in the tissues of the deficient animals examined after 10 days was considerably increased as a rule, in contrast with the observations at 23 days (31). The increase amounted to 30–80 per cent and was statistically significant for most of the tissues including blood, liver, spleen, kidney, intestine, brain, skin and muscle. The other tissues—notably cartilage and intervertebral disc—showed smaller increments of radioactivity. Cartilage behaved differently from the other tissues, showing a lowered uptake in series 1. The incorporation of radiosulfate into CSA was also diminished in series 1, in comparison to CSA from normal animals. It will further be seen from the table that the ratios of deficient to normal (def/nor) agreed well between the two series, although they were frequently a little higher in the second series. The def/nor ratios of CSA showed reasonable agreement with the corresponding values of cartilage, averaging 1.1.

Table 2 presents the regression analysis of the data in Table 1. Nearly all of the regression lines had a positive slope ($b > 0$). The following tissues generally showed a significant correlation to liver uptake: blood, spleen, kidney, intestine, brain and skin. The radioactivity of cartilage and intervertebral disc was poorly correlated to

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Table 1. Mean radioactivities of tissues 48 hours after the administration of radiosulfate to normal and deficient animals, after 10 days on the scorbutogenic diet.

Tissue and series	Deficient animals $n_1 = 16$ $n_2 = 9$	Normal animals $n_1 = 7$ $n_2 = 8$	Student's <i>t</i> Def. - Nor	Ratio Def/Nor
Blood				
1	326 ± 20	197 ± 14	4.0***	1.66
2	430 ± 23	222 ± 13	8.5***	1.93
Liver				
1	958 ± 55	593 ± 38	4.0***	1.61
2	1291 ± 45	713 ± 47	8.5***	1.81
Spleen				
1	836 ± 36	536 ± 23	5.3***	1.56
2	1064 ± 56	609 ± 31	6.9***	1.75
Kidney				
1	915 ± 48	582 ± 31	4.3***	1.57
2	1159 ± 24	650 ± 40	11.2***	1.78
Intestine				
1	1290 ± 91	834 ± 62	3.1**	1.55
2	1752 ± 102	1063 ± 92	5.3***	1.65
Brain				
1	196 ± 11	119 ± 3	4.2***	1.64
2	235 ± 8	142 ± 9	7.7***	1.65
Skin				
1	426 ± 38	285 ± 14	2.3*	1.49
2	542 ± 47	341 ± 36	3.3**	1.59
Muscle				
1	176 ± 10	119 ± 6	3.6**	1.48
2	233 ± 14	182 ± 9	3.2**	1.28
Bone				
1	589 ± 48	363 ± 46	2.8*	1.62
2	374 ± 32	289 ± 27	2.0	1.29
Teeth				
1	192 ± 15	139 ± 19	2.0	1.38
2	203 ± 17	182 ± 21	0.8	1.12
Cartilago				
1	644 ± 49	1074 ± 69	4.8***	0.60
2	1598 ± 145	1128 ± 86	2.7*	1.42
Pooled CSA				
1	10220	11450	—	0.89
2	12530	9690	—	1.29
Interv. disc				
1	1346 ± 75	1238 ± 55	0.9	1.09
2	1683 ± 88	1294 ± 35	3.9**	1.30

Table 2. Regression analysis of the data in Table 1.

Tissue and series	Deficient animals			Normal animals		
	$\bar{y}$	ϱ	$y = a + bx$	$\bar{y}$	ϱ	$y = a + bx$
Blood						
1	0.34	0.78***	$0.06 + 0.28x$	0.34	0.91**	$-0.01 + 0.35x$
2	0.34	0.35	$0.16 + 0.18x$	0.31	0.95***	$0.04 + 0.27x$
Spleen						
1	0.89	0.89***	$0.22 + 0.67x$	0.90	0.65	$0.43 + 0.47x$
2	0.83	0.73*	$-0.08 + 0.91x$	0.85	0.42	$0.27 + 0.58x$
Kidney						
1	0.95	0.92***	$0.16 + 0.79x$	0.99	0.69	$0.42 + 0.57x$
2	0.90	0.82**	$0.46 + 0.44x$	0.91	0.98***	$0.09 + 0.82x$
Intestine						
1	1.34	0.95***	$-0.16 + 1.50$	1.41	0.97***	$-0.18 + 1.59x$
2	1.36	0.47	$0.30 + 1.06x$	1.49	0.88**	$-0.23 + 1.72x$
Brain						
1	0.20	0.87***	$0.02 + 0.18x$	0.20	0.76*	$0.13 + 0.07x$
2	0.19	0.73*	$0.05 + 0.14x$	0.20	0.89**	$0.03 + 0.17x$
Skin						
1	0.45	0.76***	$-0.08 + 0.53x$	0.48	-0.13	$0.53 - 0.05x$
2	0.42	0.28	$0.13 + 0.29x$	0.47	0.89**	$-0.20 + 0.67x$
Muscle						
1	0.19	0.56*	$0.09 + 0.10x$	0.20	0.21	$0.17 + 0.03x$
2	0.18	0.50	$0.03 + 0.15x$	0.25	0.83*	$0.10 + 0.15x$
Bone						
1	0.61	0.35	$0.31 + 0.30x$	0.62	0.62	$-0.14 + 0.76x$
2	0.29	0.48	$-0.05 + 0.34x$	0.40	0.39	$0.18 + 0.22x$
Teeth						
1	0.20	0.56*	$0.05 + 0.15x$	0.24	0.62	$-0.07 + 0.31x$
2	0.15	-0.15	$0.21 - 0.06x$	0.26	0.73*	$-0.06 + 0.32x$
Cartilage						
1	0.68	0.26	$0.45 + 0.23x$	1.81	-0.48	$2.70 - 0.89x$
2	1.24	0.49	$-0.35 + 1.59x$	1.58	0.66	$0.37 + 1.21x$
Interv. disc						
1	1.40	0.59*	$0.61 + 0.79x$	2.09	-0.27	$2.48 - 0.39x$
2	1.30	0.45	$0.43 + 0.87x$	1.87	0.63	$1.40 + 0.47x$

that of liver. The regression lines of these tissues showed little concordance between series 1 and 2. The mean radioactivities of the tissues in relation to liver ($\bar{y}$) showed a close agreement between the two series and only small differences between the deficient and the normal animals. However, the radioactivities of cartilage and intervertebral disc in relation to liver were reduced by about 45 per cent and 30 per cent respectively, in the depleted animals.

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Table 3. Mean radioactivities of tissues 48 hours after the administration of radiosulfate to normal and deficient animals, after 17 days on the scorbutogenic diet. Cf. Table 4.

Tissue and series	Deficient animals $n_1 = 15$ $n_2 = 11$	Normal animals $n_1 = 7$ $n_2 = 12$	Student's t Def - Nor	Ratio Def/Nor
Blood				
1	216 $\pm$ 22	176 $\pm$ 18	1.2	1.22
2	317 $\pm$ 55	337 $\pm$ 32	0.3	0.94
Liver				
1	518 $\pm$ 41	415 $\pm$ 36	1.6	1.25
2	761 $\pm$ 136	827 $\pm$ 72	0.4	0.92
Spleen				
1	491 $\pm$ 31	480 $\pm$ 37	0.2	1.02
2	733 $\pm$ 110	783 $\pm$ 35	0.5	0.94
Kidney				
1	495 $\pm$ 33	490 $\pm$ 33	0.1	1.01
2	800 $\pm$ 108	804 $\pm$ 40	< 0.1	0.99
Intestine				
1	661 $\pm$ 61	607 $\pm$ 79	0.5	1.09
2	1145 $\pm$ 186	1240 $\pm$ 94	0.5	0.92
Brain				
1	91 $\pm$ 5	93 $\pm$ 11	0.2	0.98
2	142 $\pm$ 22	159 $\pm$ 11	0.7	0.89
Skin				
1	180 $\pm$ 12	234 $\pm$ 21	2.4*	0.77
2	249 $\pm$ 42	301 $\pm$ 26	1.1	0.83
Muscle				
1	73 $\pm$ 6	139 $\pm$ 23	3.8**	0.53
2	114 $\pm$ 18	194 $\pm$ 17	3.3**	0.59
Bone				
1	215 $\pm$ 21	200 $\pm$ 18	0.4	1.07
2	435 $\pm$ 35	466 $\pm$ 36	0.6	0.93
Teeth				
1	127 $\pm$ 14	119 $\pm$ 15	0.4	1.07
2	164 $\pm$ 18	151 $\pm$ 15	0.6	1.08
Cartilage				
1	1069 $\pm$ 141	1325 $\pm$ 19	1.1	0.81
2	1068 $\pm$ 137	1073 $\pm$ 98	< 0.1	0.99
Pooled CSA				
1	8110	10300	—	0.79
2	7770	8590	—	0.90
Interv. Disc				
1	893 $\pm$ 83	1163 $\pm$ 112	1.9	0.77
2	1007 $\pm$ 100	1262 $\pm$ 112	1.7	0.80

Table 4. Regression analysis of the data in Table 3.

Tissue and series	Deficient animals			Normal animals		
	$\bar{y}$	ϱ	$y = a + bx$	$\bar{y}$	ϱ	$y = a + bx$
Blood						
1	0.41	0.73**	$0.02 + 0.39x$	0.42	0.96***	$-0.03 + 0.45x$
2	0.43	0.95***	$0.04 + 0.39x$	0.41	0.08	$0.37 + 0.04x$
Spleen						
1	0.95	0.86***	$0.31 + 0.64x$	1.16	0.83*	$0.30 + 0.86x$
2	0.97	0.95***	$0.19 + 0.78x$	0.95	0.78**	$0.60 + 0.35x$
Kidney						
1	0.95	0.58*	$0.48 + 0.47x$	1.19	0.98***	$0.28 + 0.91x$
2	1.05	0.95***	$0.29 + 0.76x$	0.98	0.91***	$0.47 + 0.51x$
Intestine						
1	1.28	0.54*	$0.50 + 0.78x$	1.47	0.87**	$-0.45 + 1.92x$
2	1.51	0.95***	$0.21 + 1.30x$	1.50	0.86***	$0.38 + 1.12x$
Brain						
1	0.18	0.92***	$0.07 + 0.11x$	0.23	0.33	$0.13 + 0.10$
2	0.19	0.94***	$0.03 + 0.16x$	0.19x	0.85***	$0.06 + 0.13x$
Skin						
1	0.35	0.23	$0.28 + 0.07x$	0.57	0.51	$0.27 + 0.30x$
2	0.32	0.80**	$0.10 + 0.22x$	0.37	0.77**	$0.09 + 0.28x$
Muscle						
1	0.14	0.58*	$0.06 + 0.08x$	0.33	0.21	$0.20 + 0.13x$
2	0.15	0.80**	$0.05 + 0.10x$	0.23	0.73**	$0.06 + 0.17x$
Bone						
1	0.41	0.35	$0.23 + 0.18x$	0.49	0.85*	$0.05 + 0.44x$
2	0.57	0.50	$0.44 + 0.13x$	0.56	0.43	$0.34 + 0.22x$
Teeth						
1	0.24	0.59*	$0.04 + 0.20x$	0.28	0.87*	$-0.07 + 0.35$
2	0.22	0.81**	$0.11 + 0.11x$	0.18	-0.10	$0.20 - 0.02x$
Cartilage						
1	2.06	0.23	$1.36 + 0.70x$	3.19	0.40	$1.07 + 2.12x$
2	1.40	0.43	$0.97 + 0.43x$	1.30	0.66*	$0.41 + 0.89x$
Interv. Disc						
1	1.72	0.22	$1.27 + 0.45x$	2.81	0.81*	$0.31 + 2.50x$
2	1.33	0.53	$0.93 + 0.40x$	1.53	0.70*	$0.44 + 1.09x$

Seventeen days

Table 3 reveals that in contrast to the findings after 10 days, there was but little difference in radioactivity between the deficient and the normal guinea pigs after 17 days. The def/nor ratios were generally close to one in both series. In some of the deficient tissues, however, the radioactivities were lowered, in comparison with the normal tissues. The decrease amounted to 10–20 per cent for skin, cartilage and

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Table 5. Mean radioactivities of tissues 48 hours after the administration of radiosulfate to normal and deficient animals, after 23 days on the scorbutogenic diet. Cf. Table 6.

Tissue and series	Deficient animals $n_1 = 14$ $n_2 = 9$	Normal animals $n_1 = 6$ $n_2 = 12$	Student's <i>t</i> Def - Nor	Ratio Def/Nor
Blood				
1	192 ± 27	289 ± 27	2.2*	0.67
2	182 ± 40	404 ± 53	3.2**	0.45
Liver				
1	317 ± 47	884 ± 62	6.9***	0.36
2	492 ± 148	1315 ± 158	3.7**	0.37
Spleen				
1	344 ± 45	880 ± 21	6.6***	0.39
2	381 ± 89	1120 ± 105	5.1***	0.34
Kidney				
1	520 ± 49	848 ± 50	4.0***	0.61
2	555 ± 103	1211 ± 113	6.7***	0.46
Intestine				
1	758 ± 187	1333 ± 76	2.0	0.57
2	712 ± 173	1873 ± 239	3.7**	0.38
Brain				
1	65 ± 9	179 ± 16	6.8***	0.36
2	76 ± 16	230 ± 22	5.4***	0.33
Skin				
1	177 ± 28	386 ± 37	4.2***	0.46
2	122 ± 20	393 ± 37	6.2***	0.31
Muscle				
1	102 ± 12	225 ± 13	6.1***	0.45
2	79 ± 8	282 ± 20	8.3***	0.28
Bone				
1	373 ± 36	545 ± 83	2.3*	0.68
2	396 ± 33	708 ± 68	3.7**	0.56
Teeth				
1	132 ± 13	173 ± 28	1.5	0.76
2	129 ± 27	211 ± 30	2.0	0.61
Cartilage				
1	626 ± 93	1332 ± 54	4.8***	0.47
2	395 ± 53	1431 ± 115	7.3***	0.28
Pooled CSA				
1	4780	13390	—	0.36
2	2490	13770	—	0.18
Interv. Disc				
1	624 ± 79	1594 ± 139	6.5***	0.39
2	579 ± 78	1898 ± 163	6.8***	0.31

Table 6. Regression analysis of the data in Table 5.

Tissue and series	Deficient animals			Normal animals		
	$\bar{y}$	ϱ	$y = a + bx$	$\bar{y}$	ϱ	$y = a + bx$
Blood						
1	0.61	0.85***	$0.12 + 0.48x$	0.33	0.75	$0.00 + 0.33x$
2	0.37	0.97***	$0.11 + 0.26x$	0.31	0.42	$0.17 + 0.14x$
Spleen						
1	1.09	0.93***	$0.20 + 0.89x$	1.00	0.87*	$0.00 + 1.00x$
2	0.77	1.00***	$0.18 + 0.60x$	0.85	0.88***	$0.27 + 0.59x$
Kidney						
1	1.64	0.87***	$0.82 + 0.82x$	0.96	0.83*	$0.30 + 0.66x$
2	1.13	0.93***	$0.48 + 0.65x$	0.93	0.96***	$0.24 + 0.69x$
Intestine						
1	2.39	0.24	$1.27 + 1.12x$	1.51	0.79	$0.54 + 0.97x$
2	1.45	0.99***	$0.30 + 1.15x$	1.43	0.96***	$-0.03 + 1.46x$
Brain						
1	0.21	0.83***	$0.05 + 0.15x$	0.20	0.82*	$-0.01 + 0.21x$
2	0.15	0.99***	$0.05 + 0.10x$	0.18	0.92***	$0.05 + 0.13x$
Skin						
1	0.56	0.10	$0.50 + 0.06x$	0.43	0.86*	$0.04 + 0.39x$
2	0.25	0.87**	$0.13 + 0.12x$	0.30	0.24	$0.15 + 0.15x$
Muscle						
1	0.32	0.17	$0.28 + 0.04x$	0.26	0.49	$0.15 + 0.10x$
2	0.16	0.88**	$0.11 + 0.05x$	0.21	0.41	$0.16 + 0.05x$
Bone						
1	1.18	0.56*	$0.75 + 0.43x$	0.65	0.47	$-0.02 + 0.63x$
2	0.80	0.82**	$0.63 + 0.18x$	0.54	0.39	$0.37 + 0.17x$
Teeth						
1	0.42	0.53	$0.27 + 0.15x$	0.20	0.11	$0.15 + 0.05x$
2	0.26	-0.22	$0.30 - 0.04x$	0.16	0.95***	$-0.02 + 0.18x$
Cartilage						
1	1.98	0.71**	$0.57 + 1.41x$	1.51	0.57	$1.01 + 0.50x$
2	0.80	0.77*	$0.53 + 0.28x$	1.09	0.11	$1.01 + 0.08x$
Interv. Disc						
1	1.97	0.71**	$0.79 + 1.18x$	1.80	0.15	$1.46 + 0.34x$
2	1.18	0.85**	$0.73 + 0.44x$	1.44	0.28	$1.18 + 0.26x$

intervertebral disc, but was not statistically significant for these tissues. The def/nor ratios of CSA averaged 0.85. The uptake of S^{35} in muscle was significantly reduced by 45 per cent. It will be seen from Table 4 that the radioactivity of muscle in relation to liver ($\bar{x}$) was reduced to the same extent. For the other tissues the radioactivities in relation to liver were much the same in the deficient and in the normal animals.

The correlation analysis gave positive slopes for all but one of the regression lines. Significant correlations to liver uptake were frequently obtained for blood, spleen, kidney, intestine, brain, skin and muscle. Poor correlations to liver radioactivity were shown by bone, cartilage and intervertebral disc.

Twenty-three days

Table 5 discloses that the deficient animals sacrificed at 23 days, when they were severely scorbutic, had a low radioactivity in all tissues examined. All but three of the differences of the means were statistically significant. The def/nor ratios were reduced to about 0.45 with the majority of the tissues within the range of 0.35 to 0.55. The lowest value, 0.27, was encountered in CSA. The ratios were usually a little higher in series 1. The uptake of S^{35} by bone and teeth, however, was not so markedly reduced, and only bone showed significant differences between scorbutic and normal animals. For the mineralized tissues, the def/nor ratio was about 0.65.

The correlation analysis, set forth in Table 6, gave positive slopes for all but one of the regression lines. A significant correlation to liver uptake of S^{35} was generally obtained for the following tissues: blood, spleen, kidney, intestine and brain. Such a correlation was also more frequent for the scorbutic tissues. It is further seen from the tables that the mean radioactivity in relation to liver ($\bar{y}$) varied considerably between series 1 and 2 for the deficient animals. The $\bar{y}$ values for kidney, bone and teeth were increased by 40–60 per cent in the scorbutic animals.

B. Uptake of radiophosphate

The uptake of radiophosphate was examined at one point only, viz. after 24 days. One group comprising 27 surviving scorbutic guinea pigs and one group of 28 normal controls were used. There was no further subdivision of the groups. Six scorbutic (15 per cent) and three normal animals died during the experiment and were discarded. The mean weight curves of the two groups are presented in Fig. 3. At the outset of the experiment the average weight was 233 g. The deficient animals began to lose weight after approximately 17 days on the scorbutogenic diet.

The results of the radio-assays were subjected to the same statistical treatment as in experiment A. The mean radioactivities of the tissues are presented in Table 7. The uptake of P^{32} varied between the individual animals of a group to almost the

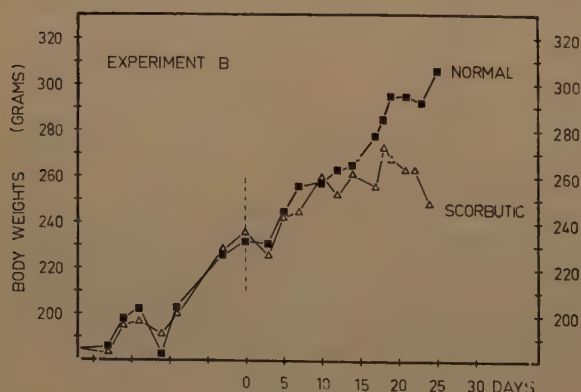


Fig. 3. Mean weight curves for experiment B. Weight at start of experiment around 253 g. Coefficients of variation about 15 per cent; somewhat higher for the scorbutic animals at end of experiment. The weight loss began after approximately 17 days.

Table 7. Mean radioactivities of tissues 48 hours after the administration of radio-phosphate to normal and deficient animals, after 24 days on the scorbutogenic diet. Cf. Table 8.

	Deficient animals <i>n</i> = 27	Normal animals <i>n</i> = 28	Student's <i>t</i> Def - Nor	Ratio Def/Nor
Blood	11.7 ± 0.3	10.2 ± 0.4	3.1**	1.15
Liver	66.3 ± 1.9	56.1 ± 2.3	3.5**	1.18
Spleen	75.3 ± 2.5	62.7 ± 3.3	3.0**	1.20
Kidney	61.2 ± 1.6	51.0 ± 4.1	3.8***	1.20
Brain	10.5 ± 0.4	7.9 ± 0.4	5.0***	1.34
Skin	12.3 ± 0.7	13.1 ± 0.8	0.8	0.93
Muscle	26.6 ± 1.3	27.8 ± 1.7	0.6	0.96
Bone	59.3 ± 3.2	79.4 ± 4.8	3.5**	0.75
Teeth	34.4 ± 2.3	72.5 ± 5.4	6.6***	0.47
Cartilage	41.1 ± 2.0	33.7 ± 1.8	9.0***	1.23

same extent as with radiosulfate. The coefficients of variation averaged about 25 per cent. There was a significantly increased radioactivity in the following tissues of the scorbutic animals: blood, liver, spleen, kidney, brain and cartilage. The def/nor ratios were about 1.2 for these tissues. The uptake of P^{32} in skin and muscle differed but little between scorbutic and normal animals. The radioactivities of bone and teeth were significantly reduced in the scorbutic guinea pigs. The def/nor ratios of the mineralized tissues were 0.75 and 0.47 respectively.

The correlation analysis of the tissue radioactivities will be found in Table 8. All regression lines had positive slopes. Significant correlations to liver radioactivity were obtained for all normal tissues except bone and for all scorbutic soft tissues except muscle. Of the mineralized tissues only normal teeth had an uptake significantly correlated to that of liver.

The mean radioactivities of the normal tissues ($\bar{y}$) varied from about 0.2 for blood, brain and skin up to 1.3–1.4 for teeth and bone. The radioactivities, in relation to liver, of bone and teeth of the scorbutic animals were considerably reduced.

Table 8. Regression analysis of the data in Table 7.

Tissues	Deficient animals			Normal animals		
	$\bar{y}$	ρ	$y = a + bx$	$\bar{y}$	ρ	$y = a + bx$
Blood	0.18	0.56**	0.09 + 0.08x	0.18	0.74***	0.06 + 1.12x
Spleen	1.14	0.67***	0.26 + 0.88x	1.12	0.78***	-0.02 + 1.14x
Kidney	0.93	0.86***	0.18 + 0.75x	0.91	0.83***	0.13 + 0.78x
Brain	0.16	0.52**	0.05 + 0.11x	0.14	0.71***	0.03 + 0.11x
Skin	0.19	0.53**	0.00 + 0.19x	0.23	0.61***	0.03 + 0.21x
Muscle	0.40	0.14	0.10 + 0.30	0.50	0.71***	-0.02 + 0.52x
Bone	0.90	0.26	0.45 + 0.45x	1.42	0.40*	0.57 + 0.84x
Inc. teeth	0.52	0.07	0.44 + 0.08x	1.29	0.63***	-0.19 + 1.48x
Cartilage	0.63	0.48**	0.10 + 0.53x	0.60	0.55**	0.18 + 0.42x

Discussion

The experimental diet was mixed from traditional ingredients. On this diet alone the guinea pigs rapidly acquired hypovitaminosis C with scurvy, as revealed by the weight curves, the blood ascorbic acid values and the X-ray examinations. In the controls the ration sufficed for rapid growth, when adequately supplemented with vitamin C. In experiment B the weight curves for the scorbutic group did not decrease as much as in experiment A, but the ultimate difference in mean weight between the normal and the scorbutic animals was statistically significant, and at the necropsies typical hemorrhages and other signs were observed in the depleted animals.

Before discussing the significance of the changes in radiosulfate uptake that were brought about by the deficiency in experiment A, some comments are required on radiosulfate metabolism in general. When radiosulfate is given by the intra-peritoneal route, it is rapidly taken up by the blood stream and dispersed in the body. Reference has already been made to the swift excretion of the bulk of the radioisotope. From the closely corresponding data of Dziewiatkowski (20) and Everett and Simmons (27) it can be calculated that in the rat, approximately 85 per cent of the administered dose is excreted in the urine and the feces within 48 hours. About nine-tenths of that amount can be recovered in the urine. Only 15 per cent is retained in the body. Sulfate is eliminated via the kidney by glomerular filtration. Lotspeich *et al.* (14, 15) have demonstrated tubular reabsorption of sulfate in the normal dog. The reabsorptive capacity is limited, and above the plasma threshold sulfate is lost at an increasing rate. Unpublished observations by the present author have confirmed that guinea pigs excrete radiosulfate at about the same rate as rats.

Presumably the excretion as well as the retention of radiosulfate is determined integratively by several factors such as size of sulfate pool, formation of sulfate from sulfur containing amino acids, functional state of the kidneys and synthesizing activity of the tissues. It is not possible to state, at present, the extent to which the individual variables contribute to the fate of radiosulfate in the body. However, minor changes in the amount excreted would account for relatively far greater alterations in radiosulfate retention. Such changes might explain e.g. the differences in radioactivity between individual animals of a group in the present experiment.

As regards the radiosulfate retained in the body, it has been shown that there is a rapid fall in blood radioactivity following the initial peak. Biochemical investigations have demonstrated that the highest labeling is found in ester sulfates, chiefly sulfomucopolysaccharides. Autoradiographic studies on fixed and embedded material at 48 hours suggest that radiosulfate is almost exclusively confined to tissues known to contain such substances (7, 23, 51). These findings tend to indicate that radiosulfate is specifically incorporated into sulfomucopolysaccharides and other ester sulfates and that little S^{35} is to be found in other sulfur compounds or in tissues with low sulfomucopolysaccharide contents. The present investigation, however, has furnished evidence that such a concept is erroneous. In agreement with the results of Dziewiatkowski (20) and Boström (2), considerable radioactivity was found in blood. Even though some of the S^{35} may have been associated with the plasma proteins (25, 63), this still suggests that the amounts of S^{35} present as inorganic sulfate are

by no means negligible. A further finding consisted in a high radioactivity in e.g. liver, spleen and kidney, amounting to about one half of that in cartilage. In these tissues S^{35} was presumably present as inorganic sulfate and/or in compounds that are more or less quantitatively removed during the histologic procedures preceding autoradiography. Data supporting this hypothesis have been reported by Dohlman (18). It must in fact be assumed that S^{35} was present in the guinea pig tissues not only as inorganic or ester sulfur but also as neutral sulfur. Recoveries of the radioisotope from all three sulfur fractions have been reported, following administration of radiosulfate. Manifestly, some of the S^{35} remains as inorganic sulfate 48 hours after the injection, and it is known to be incorporated as such into the mineral part of bone (26). The incorporation of radiosulfate into various ester sulfates—CSA (3, 22), heparin (49), keratosulfate (59), sulfocerebrosides (36) and phenolsulfates (21, 43)—is well documented. This transfer of sulfate from inorganic to ester form was recently shown to be an enzymatic process involving the formation of active sulfate (57, 64). Incorporation into non-sulfate compounds has also been demonstrated. The utilization of radiosulfate for the synthesis of taurine is generally recognized and has been observed in the rat (4) and the chick embryo (11, 46, 47). On the other hand, opinions diverge as to the extent to which radiosulfate is incorporated into cystine and methionine. Some investigators believe that radioactive cystine and/or methionine is formed in the rumen of cows, sheep and goats (1) and in rats (15, 24, 25), rabbits (18) and laying hens (48); others however, hold that such incorporation does not take place in the tissues of rats (4, 65), rabbits (12) or chick embryos (46, 47).

The distribution of the radioisotope among the three sulfur fractions varies both in different tissues and with the time elapsing after the injection (18). That tissues take up radiosulfate in different ways is suggested by the results of the present regression analysis. Nearly all of the regression lines were found to have positive slopes, as was to be expected. However, the tissues could be divided into two groups with regard to the frequency of significant correlations to liver uptake. In the first group, comprising blood, spleen, kidney, intestine and brain, a significant correlation to liver radioactivity was the rule; in the second group it was less frequent. The latter consisted of skin, muscle, bone, teeth, cartilage and intervertebral disc. In performing the regression analysis the radioactivity in liver was employed as the common denominator. There was, however, a close correlation between the radioactivities in blood and liver, respectively. Thus a significant correlation to liver uptake presumably indicates a direct dependence on the supply of radiosulfate in the blood. The highly vascularized tissues of the first group probably contained a large proportion of the radioisotope as inorganic sulfate or in some simple compound. In the "mesenchymal" tissues of the second group the incorporation of radiosulfate into more complex compounds, sulfomucopolysaccharides, as well as different vascular conditions—notably avascularity of cartilage—may, for example, have contributed to produce the apparent lack of correlation to the liver radioactivity.

The vitamin C-deficiency modified the uptake of radiosulfate by the guinea pigs in two opposite directions. In the deficient animals examined after 10 days on the scorbutogenic ration, the radioactivity of the tissues was increased, on the average, by 50 per cent. After 23 days there was a decrease of about 55 per cent. The tissue radioactivities of deficient animals examined at 17 days did not, as a rule, differ significantly from those of normal controls. The tissues examined represented about 90 per

cent of the body weight less intestinal contents. It appears that the deficient animals retained a greater portion of the administered dose at 10 days, and a smaller part at 23 days. This assumption is partly corroborated by the writer's observation that scorbutic guinea pigs had an accelerated urinary excretion of S^{35} at the latter time (unpublished). The question then arises whether the changes in tissue radioactivity were due to alterations in the elimination of S^{35} from the body, or whether the changes in excretion were caused by a modified tissue utilization of sulfate. It is difficult, at present, to reach any definite conclusion regarding the many factors involved. Caution is therefore required when assessing the signification of the changes in S^{35} incorporation into CSA during the development of scurvy. An important finding in this connection is that cartilage and intervertebral disc, to some extent, reacted differently from the other tissues to the depletion of ascorbic acid. The radioactivities of the cartilaginous tissues showed, on the average, only a 10 per cent increase at 10 days in comparison with 57 per cent for the remaining tissues. At 17 days cartilage and intervertebral disc radioactivities were 16 per cent below normal in contrast to 5 per cent for the other tissues; at 23 days the decrease amounted to 64 and 53 per cent, respectively. For CSA the following average def/nor ratios were obtained—1.09 at 10 days, 0.84 at 17 days and 0.27 at 23 days.

Thus, the cartilaginous tissues did not share the considerable increase in radioactivity at 10 days, and thereafter were affected earlier and more markedly than the other tissues by the hypovitaminosis C. Tentatively, the following interpretation of the results may be suggested. The increased radioactivity of the tissues at 10 days was due mainly to a slower elimination of the radioisotope from the sulfate pool and/or an accelerated incorporation into compounds other than sulfomucopolysaccharides, while there was no indication of an increased production of CSA. At 17 days the first evidence of an impaired synthesis of CSA was present, whereas at 23 days the formation of this mucopolysaccharide was considerably retarded. It cannot be ruled out, however, that a more rapid turnover of the sulfate pool contributed to the low radioactivity in the tissues at the latter time. As far as this interpretation concerns the metabolism of CSA, it is substantiated by further observations in a paper to be published (29).

Accordingly, the synthesis of CSA becomes markedly retarded only when the guinea pigs have been deprived of ascorbic acid for at least 3 weeks and are rapidly declining. It is, therefore, quite conceivable that the effects on the synthesis of CSA are due to the inanition rather than to the vitamin C deficiency itself.

An interesting observation is that not only the cartilaginous tissues but also muscle behaved differently from the other tissues. A significant 45 per cent decrease in muscle uptake of S^{35} was already found at 17 days in the deficient guinea pigs. At 23 days the decrease amounted to 54 per cent, i.e., almost 10 per cent more than the average for the scorbutic tissues in general. Furthermore, muscle did not show the 20 per cent increase in P^{32} uptake exhibited by most of the scorbutic soft tissues. It seems reasonable to link these divergences to the scorbutic myopathy, characterized histologically by hemorrhages, fragmentation, giant cell accumulation and calcification, and biochemically among other things by a decrease in the content of glutamine (13) and various phosphorus compounds (37). Muscles are affected by the deficiency almost as early as are the teeth.

The following observations, though not entirely comparable with those reported here, corroborate to some extent the present findings and interpretations. Kodicek and Loewi (42) found *in vivo* and *in vitro*, a decreased uptake of radiosulfate in regenerating tendons of scorbutic guinea pigs examined at 19–22 days. On the other hand, no decrease in liver radioactivity was observed *in vivo*. A conceivable explanation is that their guinea pigs were less scorbutic. Besides being examined one to four days earlier, the animals were also a little heavier, and hence succumbed more slowly to the deficiency (56). Kodicek and Loewi washed their specimens in ice-cold Ringer's solution for 1 hour prior to incineration and radio-assay. This may have helped to produce their divergent results.

Upton and Odell (66) found a plasma level of radiosulfate that was about 30 per cent below normal in scorbutic guinea pigs examined at 17 to 22 days. The uptake of radiosulfate in blood platelets was similarly reduced. Autoradiograms showed the uptake of S^{35} of healing wounds to be reduced in the deficient animals.

The influence of ascorbic acid depletion on the uptake of radiosulfate in healing wounds has also been studied by Moltke (50). He found that the radioactivity in the intact skin of deficient animals was about 40 per cent lower than in normal controls after only 14 days on the scorbutogenic regimen. The amount of S^{35} in skin wounds was also reduced. However, the wound/skin ratio of radioactivity was about 16 per cent higher in the depleted animals. This was taken to imply a better utilization of available sulfate in scorbutic animals, on the assumption that all S^{35} was present in sulfomucopolysaccharides.

The authors cited above apparently did not consider the possibility that the reduced uptake of S^{35} in vitamin C-deficient animals may not truly reflect changes in sulfomucopolysaccharide metabolism. Importance therefore attaches to the observation made by Kodicek and Loewi (42) that the uptake of S^{35} by scorbutic granulation tissues *in vitro* was also reduced.

In experiment B the uptake of radiophosphate was found to be affected by vitamin C-deficiency in a manner differing from that of radiosulfate. In most of the scorbutic soft tissues there was a 20 per cent increase in radioactivity due to P^{32} . In bone and teeth a 25–50 per cent decrease of radioactivity was observed. These results are consistent with the findings reported previously (31). The biologic significance of the increased uptake of radiophosphate in the soft tissues is not clear, for much the same variety of factors has to be considered as with radiosulfate, but the observation is of interest relative to the uptake of P^{32} in bone and teeth. In the mineralized tissues there is a preponderance of inorganic constituents over organic matter. There is, accordingly, reason to believe that the uptake of P^{32} in bone and teeth reflects essentially an incorporation into and/or adsorption to the mineral compartment, although this isotope is known to be incorporated into a multitude of organic compounds. It seems justifiable to conclude from the present experiment that there is a diminished deposition of bone salt in advanced acute scorbutus. Microscopically there is evidence suggesting a reduction in intramembranous and endochondral ossification (35). It has not heretofore been shown, however, with any assurance to what extent ascorbic acid influences the metabolism of the bone minerals. Indeed, the literature on this subject—recently reviewed by Bourne (9) and Reid (56)—is highly controversial.

Salter and Aub (61) found that the long bones of scorbutic guinea pigs did not stain with alizarin *in vivo*. Danielli *et al.* (17) and Bourne (8) demonstrated in vitamin C-deficiency a reduced production of the enzyme, alkaline phosphatase, associated with osteoblastic activity. Humphreys and Zilva (39) reported that no significant changes in blood level of calcium and phosphorus took place during the development of scurvy, although the retention of these mineral elements was reduced after about 15 days on the deficient diet.

A comparison of the uptake of S^{35} and P^{32} , respectively, by the different tissues in relation to liver ($\bar{y}$), amply illustrates the separate pathways of the two radioisotopes in the body. The highest uptake of S^{35} occurred in cartilage and intervertebral disc—tissues rich in sulfomucopolysaccharides. With P^{32} the greatest radioactivities were found in the mineralized tissues, bone and teeth. The uptake ratios of S^{35} to P^{32} , in relation to liver, were approximately 2:1 for blood, 3:1 for cartilage, 1:2 for muscle, 1:3 for bone and 1:6 for teeth.

SUMMARY

In an experiment conducted on 126 guinea pigs the radioactivities due to S^{35} —48 hours after the administration of radiosulfate—of various tissues and rib cartilage CSA were examined after 10, 17 and 23 days on a scorbutogenic ration. The tissue radioactivities of the deficient animals were, on the average, 50 per cent above normal at 10 days and 55 per cent below normal by 23 days. The synthesis of CSA did not appear to be markedly affected until after 23 days, at which time it was apparently reduced.

The radioactivities of various tissues following the administration of P^{32} phosphate were similarly determined in a second experiment, comprising 55 guinea pigs, after 24 days on the deficient diet. In the scorbutic soft tissues, the radioactivities averaged 15 per cent above normal. In the mineralized tissues, bone and teeth, the uptake of P^{32} was 25–50 per cent lower than in the normal controls.

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